Effects of Production Locations and Plucking Intervals on Clonal Tea Fatty Acids Levels in the Kenya Higlands

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Tea leaves contain unsaturated fatty acids which are precursors of aroma compounds. Despite uniform plucking practices in Kenya, plain black tea quality varies with locations. Variations in tea aroma and/or precursors with production area have not been established in a single cultivar under same agronomic input at different locations. Trials were conducted in five locations using clone BBK 35, harvested at varying intervals. The fatty acids varied (P ≤ 0.05) with locations and increased (P ≤ 0.05) with long plucking intervals, demonstrating leaf of same standard plucked at varying intervals contains different levels of fatty acids even in one location. The different increase rates in various locations might be in part the reason of the differences in aroma of black tea from different locations even when agronomic inputs are similar. Results demonstrate that harvesting at shorter intervals and developing region-specific plucking policies may improve tea quality.

Introduction

Tea (*Camellia sinensis* (L.) O. Kuntze) yields and black tea quality are influenced by agronomic practices, especially plucking (Owuor, 1996). Plucking is an expensive undertaking (Ellis and Grice, 1981) and constitute up to 80% of the total field cost of tea production (Sharma, 1987). During this process young tender shoots are harvested for processing into various tea beverages. The recommended plucking standard in tea is two leaves and a bud that gives desirable good quality teas (Othieno, 1988), although there are producers harvesting mature leaf. In practice, it is very difficult to exclusively achieve the recommended standard in commercial tea harvesting as various portions of different standards leaf are usually plucked and the amount of coarse fraction and fine fraction in the plucked crop determines the overall leaf quality.

Shoots of similar two leaves and a bud can be obtained through optimizing harvesting intervals when most leaves are in the stage of two leaves and a bud. Plucking frequency is therefore an important factor which controls the proportions of different leaf standard in the harvested crop (Odhiambo, 1989) and influences yield (Owuor *et al.*, 2009) and tea quality (Baruah *et al.*, 1986, Owuor *et al.*, 2009). Flavoury black teas are valued for their unique smell attributed to the levels and distribution of the volatile flavour compounds (VFC) (Robison and Owuor, 1992) while plain teas are valued for their taste, briskness and colour characteristics, which are influenced by non-volatile (theaflavin and thearubigin) components. The VFC in tea can further be classified into primary and secondary products (Sanderson and Graham, 1973). The primary aroma compounds are biosynthesized by the plant and are present in the fresh tea leaf, while the secondary products are derived from carotenes, amino acids, unsaturated fatty acids and other lipids (Robinson and Owuor, 1992) and terpene glycosides (Skobeleva *et al.*, 1987). The VFC contributing the green grassy aroma are dominated by products of unsaturated fatty acids in the green tea leaf (Yamanishi *et al.*, 1978, Robinson and Owuor, 1992). These VFC are composed mostly of straight chain C_6 C_7, C_9, and C_{10} aldehydes, alcohols and acids, which are products of unsaturated fatty acids breakdown during black tea processing (Hatanaka *et al.*, 1995, Kajiwara *et al.*, 1982, Sekiya *et al.*, 1984). These compounds reduce black tea

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quality (Mahanta *et al.*, 1988, Robinson and Owuor, 1992) but enhance green tea quality (Wang *et al.*, 1988). *Cis*-4,5-epoxy-E-2-decenal (Gardener and Salke, 1984, Gassenmeier and Schieberle, 1994) and *trans*-4,5-epoxy-E-2-decenal (Kumazawa *et al.*, 2006) have also been reported as products of linolenic acid degradation. The levels of these compounds usually increase with increase in amounts of unsaturated fatty acids in green tea leaves (Mahanta *et al.*, 1988). The unsaturated fatty acids in tea leaf are therefore key tea quality determinants. Although the levels of these volatile flavour compounds changed with plucking intervals in black tea in Assam, India (Baruah *et al.*, 1986), the magnitude of changes in unsaturated fatty acids levels have not been established and it is not known whether their levels change with locations as observed in plain black tea quality parameters (Owuor *et al.*, 2009, 2010a, 2010b) when the cultivar is the same. In the previous studies, the genetic make of the plants used and agronomic practices were varied. It is therefore not possible to assert if the noted variations were solely due to locations.

Plucking interval influences the aroma quality of black tea, especially, shorter plucking intervals improve the aroma quality of black tea (Baruah *et al.*, 1986, Owuor *et al.*, 2000). At single sites, the unsaturated fatty acids levels increased with maturity of the shoot (Bhuyan *et al.*, 1991, Owuor *et al.*, 1990) implying a tendency to produce poor flavoury black tea with coarse plucking standard. It is not known if the levels of fatty acids of a single cultivar subjected to same agronomic management and inputs will change with location of production when same plucking standard is maintained. Changes in black tea aroma with location had been reported in Assam (Mahanta *et al.*, 1988). Since there were changes in VFC it is likely the fatty acids may also vary. However, it is not known if the magnitude and pattern of the changes in fatty acids levels with location of production will be similar in one genotype under uniform management. Lack of similar magnitudes and patterns in changes may suggest need for varying plucking interval recommendations to enhance tea quality.

Materials and Methods

The trials were laid out in five main tea growing regions of Kenya at Karirana (altitude 2260 m above mean sea level (amsl), latitude 1°6'S, longitude 36°39'E), Timbilil (altitude 2180 m amsl, latitude 0°22'S, longitude 35°21'E), Changoi (altitude 1860 m amsl, latitude 0°29'S, longitude 35°14'E), Sotik Highlands (altitude 1800 m amsl, latitude 0°35'S, longitude 35°5'E) and Kipkebe (altitude 1800 m amsl, latitude 0°41'S, longitude 35°5'E). Clone BBK 35 plantations that had been uniformly managed with known past cultivation history, were selected from each site. The trials were laid

out in a randomised complete block design with three plucking intervals rates (7, 14, and 21 days) and replicated three times. The standard plucking of two leaves and a bud was done in all the plots. Each effective plot comprised 60 plants surrounded by a line of tea bushes that served as guard rows. The plots were uniformly managed and prior to and during the experiments, all the plots were receiving 150 kg N ha^{-1} year^{-1} as NPKS 25:5:5:5.

The samples were collected by plucking 100 g of two leaves and a bud from each plot. The leaves were steamed for 1 minute to deactivate the enzymes responsible for oxidative degradation reactions in tea leaves. The leaves were cooled, then oven dried 96°C to a constant weight. The dried leaves were ground to fine powder using coffee grinder. Each dried sample (10 g) was mixed with 0.015 g of heptadecanoic acid (as an internal standard) and then extracted twice with 2:1 (v/v) chloroform and methanol mixture for three hours with continuous stirring at room temperature. The mixture was shaken together with 20ml dilute potassium chloride solution. The organic layer was separated and solvent removed under reduced pressure.

The lipids were transesterified and esterified to their corresponding methyl esters (Owuor *et al.*, 1990). To the lipids were mixed with 10ml methanolic sodium hydroxide solution (0.05 mol/L) in a round bottomed flask fitted with a condenser. A small volume of tetrahydro furan was added to improve the solubility of the lipids. The mixture was refluxed for ten minutes. About 10ml of boron trifluoride-methanol complex (about 14% BF$_3$) was added and the mixture refluxed for a further two minutes. The solution was cooled to room temperature and 5ml hexane was added and the mixture refluxed again for two minutes. A saturated sodium chloride solution was added and the hexane layer was separated and dried with anhydrous sodium sulphate. The extraction was done twice and activated silica gel was added to the hexane layer with continuous stirring to remove all the chlorophyll. The silica gel was filtered off and hexane removed under reduced pressure using rotary evaporator. The fatty acid methyl esters were then dissolved in small amount of hexane in sample bottle before injection into the GC

The GC analysis was carried out under the conditions reported by Munavu (1983). The GC was fitted with fused 50 m silica gel capillary columns coated with carbowax, (film thickness; 0.20 um, 0.25 mm inner diameter) and flame ionization detector. The temperature programme was holding at 160°C for 4 min then increasing linearly to 170°C at 0.6°C/min and holding for 9 min and finally increasing to 200°C with a rate of 3.5°C per minute then holding at 200°C for another 4 min. The flow rates were 3.0 mL/min for the carrier (N$_2$), 30.0 mL/min makeup (air) gases, and 40.0 mL/min H$_2$

and 400.0 mL/min air for detector gases, respectively. The detector temperature was set at 230.0°C. Sample of 0.1 μL was injected. The compounds were identified and quantified by corroborative retention times and peak area of authentic fatty acid standards relative to internal standard.

Results and Discussion

The current plucking interval recommendation is the same for all tea growing locations in Kenya (Othieno, 1988). The fatty acids were analysed as fatty acids methyl esters (Owuor *et al.*, 1990). A typical gas chromatogram is presented in Figure 1. There is an association between the fatty acids levels in green leaf and black tea aroma compounds that are products of their degradation (Bhuyan *et al.*, 1991) during tea processing. The distribution of fatty acids due to plucking intervals in different locations is represented in Tables 1 – 3. At all plucking intervals in all locations, the levels of saturated fatty acids in the leaf varied in the order of n-hexadecanoic (palmitic) acid (C16:0) > n-octadecanoic (stearic)
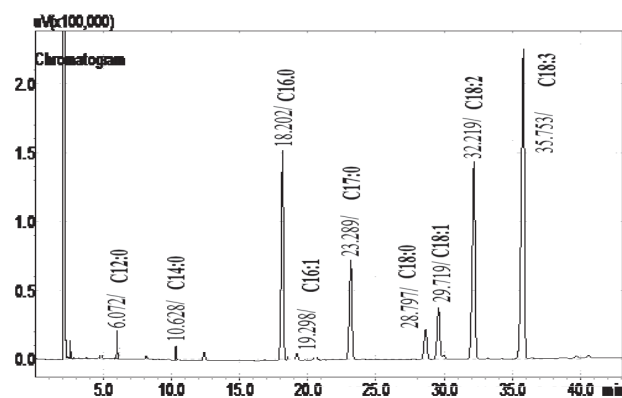

Fig. 1. Gas chromatogram of green tea leaves fatty acids methyl esters.

acid (C18:0) > n-tetradecanoic (myristic) acid (C14:0) > n-dodecanoic acid (lauric) (C12:0) in all the locations (Table 1). Longer plucking intervals resulted into high levels of the fatty acids. The levels of the individual saturated fatty acids varied ($P \leq 0.05$) with location. The extents of variations in

Table 1. The changes in saturated fatty acid (mg/g dry leaf) levels with location of production and plucking intervals.

FA	Plucking intervals	Location					Mean plucking intervals
		Karirana	Timbilil	Changoi	Sotik Highland	Kipkebe	
C12:0	7 days	0.057	0.046	0.167	0.092	0.014	0.057
	14 days	0.087	0.048	0.184	0.123	0.048	0.098
	21 Days	0.136	0.051	0.263	0.150	0.188	0.158
	Mean Location	0.093	0.049	0.205	0.122	0.083	
	CV (%)			7.34			
	LSD, $P \leq 0.05$			0.009			0.017
	Interaction, $P \leq 0.05$			0.016			
C14:0	7 days	0.145	0.062	0.130	0.111	0.255	0.141
	14 days	0.187	0.064	0.224	0.128	0.264	0.174
	21 Days	0.240	0.075	0.240	0.147	0.278	0.196
	Mean Location	0.191	0.067	0.198	0.129	0.266	
	CV (%)			3.79			
	LSD, $P \leq 0.05$			0.006			0.013
	Interaction, $P \leq 0.05$			0.012			
C16:0	7 days	1.414	1.617	1.269	1.167	1.537	1.401
	14 days	1.458	1.641	1.333	1.312	1.552	1.459
	21 Days	1.773	1.967	1.529	1.359	1.591	1.644
	Mean Location	1.548	1.742	1.377	1.279	1.560	
	CV (%)			4.33			
	LSD, $P \leq 0.05$			0.066			0.132
	Interaction, $P \leq 0.05$			0.123			
C18:0	7 days	0.622	0.488	0.872	0.222	0.447	0.570
	14 days	0.856	0.515	0.887	0.452	0.474	0.637
	21 Days	0.979	0.575	1.016	0.490	0.526	0.718
	Mean Location	0.819	0.526	0.925	0.455	0.483	
	CV (%)			1.600			
	LSD, $P \leq 0.05$			0.011			0.021
	Interaction, $P \leq 0.05$			0.020			

Table 2. The changes in unsaturated fatty acid (mg/g dry leaf) levels with location of production and plucking intervals.

FA	Plucking intervals	Location					Mean plucking intervals
		Karirana	Timbilil	Changoi	Sotik Highland	Kipkebe	
C16:1	7 days	0.042	0.029	0.038	0.056	0.026	0.038
	14 days	0.049	0.030	0.043	0.059	0.032	0.043
	21 Days	0.063	0.032	0.045	0.065	0.036	0.048
	Mean Location	0.051	0.030	0.042	0.060	0.032	
	CV (%)			6.53			
	LSD, P ≤ 0.05			0.003			0.006
	Interaction, P ≤ 0.05			0.006			
C18:1	7 days	0.361	0.375	0.494	0.301	0.485	0.403
	14 days	0.384	0.386	0.511	0.322	0.507	0.422
	21 Days	0.412	0.411	0.532	0.347	0.521	0.445
	Mean Location	0.386	0.391	0.512	0.323	0.504	
	CV (%)			1.16			
	LSD, P ≤ 0.05			0.005			0.009
	Interaction, P ≤ 0.05			0.008			
C18:2	7 days	1.617	1.761	1.226	1.565	1.428	1.520
	14 days	1.739	1.840	1.372	1.618	1.481	1.610
	21 Days	2.031	2.136	1.810	1.675	1.519	1.834
	Mean Location	1.796	1.912	1.469	1.619	1.476	
	CV (%)			0.74			
	LSD, P ≤ 0.05			0.012			0.025
	Interaction P ≤ 0.05,			0.023			
C18:3	7 days	2.859	1.990	1.659	1.943	1.618	2.014
	14 days	3.193	2.080	1.894	1.990	2.137	2.259
	21 Days	3.841	2.262	2.359	2.126	2.176	2.553
	Mean Location	3.298	2.111	1.971	2.019	1.977	
	CV (%)			0.75			
	LSD, P ≤ 0.05			0.017			0.035
	Interaction, P ≤ 0.05			0.032			
Total Unsaturated fatty acids	7 days	4.879	4.155	3.418	3.865	3.560	3.975
	14 days	5.365	4.336	3.820	3.989	4.157	4.334
	21 Days	6.347	4.840	4.746	4.213	4.254	4.880
	Mean Location	5.530	4.444	3.995	4.022	3.990	
	CV (%)			0.53			
	LSD, P≤ 0.05			0.024			0.048
	Interactions, P ≤ 0.05			0.044			

Table 3. The variation of total fatty acids (mg/g of dry leaf) due to location and plucking intervals.

FA	Plucking intervals	Location					Mean plucking intervals
		Karirana	Timbilil	Changoi	Sotik Highland	Kipkebe	
Total fatty acids	7 days	7.118	6.369	5.855	5.657	5.814	6.163
	14 days	7.957	6.604	6.449	6.005	6.495	6.702
	21 Days	9.475	7.508	7.793	6.358	6.839	7.594
	Mean Location	8.183	6.827	6.699	6.007	6.383	
	CV (%)			1.12			
	LSD, P ≤ 0.05			0.078			0.155
	Interaction, P ≤ 0.05			0.144			

the levels of the acids were not uniform. The order of concentration for n-dodecanoic acid was: Changoi > Sotik Highlands > Karirana > Kipkebe > Timbilil. For n-tetradecanoic acid the order was: Kipkebe > Changoi > Karirana > Sotik Highlands > Timbilil. The level of n-hexadecanoic acid was: Timbilil > Kipkebe > Karirana > Changoi > Sotik Highlands and the order for n-octadecanoic acid was: Changoi > Karirana > Timbilil > Kipkebe > Sotik Highlands. Although there were increases in the levels of the saturated fatty acids with plucking intervals, the rates of increases were not constant in all the locations causing significant ($P \leq 0.05$) interaction effects (Table 1). This demonstrates that similar plucking interval does not produce similar variations in levels of tea leaves saturated fatty acids in a single genotype at different locations.

The levels of the individual and total unsaturated fatty acids increased ($P \leq 0.05$) with longer plucking intervals at all locations (Tables 2 and 3), explaining in part the general black tea aroma quality declines with long plucking intervals even when plucking standard is the same (Owuor *et al.*, 2000). The magnitudes and patterns of the changes varied with sites of production. The non-uniform variations in the levels of these acids are attributable to different climatic conditions prevailing in the different regions subjecting the plants to differences in growth parameters.

An increase in unsaturated fatty acid levels with coarse plucking standard had been observed (Owuor *et al.*, 1990). A plucking interval encouraging harvesting more mature leaf is therefore expected to have higher fatty acids levels. However, in this study, the plucking standard was constant and selective. Only two leaves and a bud were plucked and the influence of coarse plucking was minimal. Despite maintaining same plucking standard, fatty acids levels still increased with long plucking intervals even at a single site. This demonstrates for the first time that within Kenya, there are significant ($P \leq 0.05$) variations in unsaturated fatty acid and total fatty acid levels in the shoots of one genotype subjected to the similar plucking management in different regions.

Significant differences ($P \leq 0.05$) in the level of unsaturated fatty acids with location at constant plucking interval were observed. (9Z, 12Z, 15Z)-octadecatrienoic (linolenic) acid ($C18:3^{\Delta 9, 12, 15}$) varied in the order Karirana > Timbilil > Sotik Highlands > Kipkebe > Changoi. The variation in (9Z, 12Z)-octadecadienoic (linoleic) acid ($18:2^{\Delta 9, 12}$) was significant ($P \leq 0.05$) in all locations such that Timbilil > Karirana > Sotik Highlands > Kipkebe > Changoi. (9Z)-octadecaenoic (oleic) acid ($18:1^{\Delta 9}$) varied in the order Changoi > Kipkebe > Timbilil > Karirana > Sotik Highlands and (9Z)-hexadecaenoic (palmitoleic) acid ($C16:1^{\Delta 9}$) varied in the order Sotik Highlands > Karirana > Changoi > Kipkebe > Timbilil (Table

2). Total unsaturated fatty acid and total fatty acid levels varied significantly with location in the order Karirana > Timbilil > Sotik Highlands > Changoi > Kipkebe (Table 3). Thus, similar plucking intervals will not produce similar levels of precursors of aroma quality in parameters all areas even in one genotype, partly explaining the differences observed in black teas VFC grown in Kenya and Malawi (Owuor *et al.*, 2008). It is important therefore that plucking management is domesticated in the regions of intended production.

Although there were significant ($P \leq 0.05$) increases in fatty acid levels with increase in plucking intervals, the increase rates were not constant in all the locations causing significant interactions effects ($P \leq 0.05$) (Table 2 and 3). Therefore, similar plucking intervals would lead to different levels of precursors of aroma quality parameters in different regions causing differences in tea quality. This is attributable to different growth rates in different locations (Ng'etich *et al.*, 2001) leading to achievement of the recommended two leaves and a bud (Othieno, 1988) after different time lengths in different locations. Therefore the uniform blanket recommended plucking interval throughout Kenya (Othieno, 1988) may be subjecting some areas to low quality production. Studies on plain quality parameters on (Owuor *et al.*, 2010a, 2010b, 2009) showed same trend. This implies that each location has an optimal plucking management practice. There is need to intensify the development of region specific agronomic input levels to improve tea quality.

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