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Plucking Standard Effects and the Distribution of Fatty Acids in the Tea (*Camellia sinensis* (L.)) Leaves

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ABSTRACT

The order of occurrence of fatty acids (FA) in the leaves and stem of tea shoots is linolenic acid > linoleic acid > palmitic acid > stearic acid > oleic acid > palmitoleic acid, irrespective of the plucking standard or portion of the shoot. The stem had the least FA levels. Linolenic acid dominated the FA and increased with maturity of the leaf and coarser plucking standard. Similarly, total FA, total unsaturated FA and linolenic plus linoleic acids increased with coarse plucking standard and maturity of the leaf. This explains quality deterioration due to the higher amount of the group I volatile flavour compounds arising from the coarse plucking standard.

INTRODUCTION

The young tender shoots of tea, *Camellia sinensis* (L.) O. Kuntze, are used in the manufacture of the beverage black tea. To make good black tea, the recommended plucking standard in most countries is two leaves and a bud. However, because of higher crop per plucking round obtained with coarse plucking standards, it is known that, occasionally, some producers have practised coarser plucking standards than those recommended. In Kenya, ensuring that the correct plucking standard is practised is a problem, since the pluckers are paid according to the weight of the shoots plucked. In the small scale farmers sector, the farmers are also paid on the basis of the weight

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of leaves delivered. There is therefore a tendency for a coarser plucking standard than the recommended practice.

However, it has been argued that a coarse plucking standard reduces black tea quality and this was recently practically demonstrated (Owuor *et al.* 1987*a*). It was shown that caffeine, theaflavins, Group II volatile flavour compounds (VFC), i.e. VFC responsible for sweet aroma of tea, and flavour index, decrease while Group I VFC, i.e. VFC responsible for poor greenish, unpleasant flavour in tea, increase with coarse plucking standard (Owuor *et al.* 1987*a*). More recently, Mahanta *et al.* (1988) made a similar observation on the VFC of Assam black teas. Thus reduction of black tea quality with coarse plucking standard occurs wherever it is practised.

The group I VFC are products of lipid breakdown during the manufacture of black tea (Yamanishi, 1981). Indeed, Hatanaka *et al.* (1987 and references therein) demonstrated that linolenic acid, via lipoxygenase initiated regio- and enantio-selective hydroperoxidation (Kajiwara *et al.* 1982) breaks down to form Z-3-hexenal which partly reduces to Z-3-hexenol, and partly isomerizes to E-2-hexenal also reducing to E-2-hexenol. These C6 unsaturated aldehydes and alcohols dominate the group I VFC and are closely followed by hexanal and *n*-hexanol, which are products of linoleic acid breakdown following the same mechanism. In the same manner, nonanal and *n*-nonanol, heptanal and *n*-heptanol are produced from oleic acid and palmitoleic acid, respectively. These VFC, which are products of fatty acid (FA) breakdown during black tea manufacture, comprise over 90% group I VFC in black tea (Owuor *et al.*, 1987*b*, 1988; Horita & Owuor, 1987). The FA are therefore important quality indices in green leaf as their levels determine the flavour quality of tea. Although the sum of group I VFC had been shown to increase with coarse plucking standard, it had not been established whether the increase was due to the increase in the precursor compounds (FA) or the enzyme activity responsible for the breakdown. This study was therefore carried out to determine how plucking standards affect the FA levels in the green leaf. The study was also undertaken to establish how the FA are distributed in the different parts of the young shoots of tea.

MATERIALS AND METHODS

Tea leaves

The tea shoots, used to determine the distribution of the FA in different parts of young shoots, were obtained from the mother bushes of the clonal multiplication plots at the Tea Research Foundation of Kenya (TRFK) (altitude 2178 m above mean sea level and longitude 0°22′ South). The shoots

were obtained from clone 6/8. The shoots were then divided into bud, 1st leaf, 2nd leaf, 3rd leaf, 4th leaf, 5th leaf, 6th leaf and stem (between bud and 6th leaf). The mother bushes had received 200 kg N/ha/year as NPKS 25:5:5:5 fertiliser. Sampling was done in triplicate. Clones 6/8 and 31/8 were coarsely plucked from a TRFK Clonal Field Trial, set up in 1982 to determine effect of plucking standard on FA levels in tea shoots. The plucked shoots were further divided into bud, one leaf and a bud, two leaves and a bud and three leaves and a bud. The teas were receiving 150 kg N/ha/year as NPKS 25:5:5:5. Sampling was done in triplicate.

Treatment of the leaf

Within an hour after plucking, the leaves were steamed for 1 min to deactivate lipoxygenase. The leaves were then oven-dried at 96°C for 4 h. The dried leaves were ground to powder using a coffee grinder.

The powder was weighed (10 g) plus 0·15 g heptadecanoic acid (C17:0) (internal standard), then extracted twice with 2:1 (v/v) chloroform and methanol mixture for 3 h with continuous stirring at room temperature. The mixture was shaken with 20 ml dilute potassium chloride solution. The organic layer was separated and solvent removed using a rotatory evaporator. The lipids were then transesterified or esterified to their methyl esters as follows: to the lipid mixture in a round-bottom flask, fitted with a condenser, was added 10 ml of 0·5N methanolic sodium hydroxide solution. A small volume of tetrahydrofuran was added to effect solubility of the lipids. The mixture was then refluxed for 10 min. About 10 ml of boron trifluoride–methanol complex (about 14% BF_3) was added to the mixture and the mixture was refluxed for a further 2 min. The solution was cooled to room temperature followed by addition of 5 ml hexane. The mixture was boiled again for 2 min. A saturated sodium chloride solution was added and the hexane layer was separated into a container with anhydrous sodium sulphate. The extraction was done twice. To the hexane layer was added activated silica gel with continuous stirring until all the chlorophyll was removed from the solution. The silica gel was then filtered off and hexane removed under reduced pressure on the rotatory evaporator. The fatty acid methyl esters (FAMES), in a small amount of hexane, were stored in a sample bottle. GC analysis was done under the conditions of Munavu (1983). Samples were identified by corroborative retention times on the GC of authentic samples.

Chemical and statistical analysis

A randomised complete block design program was used to do analysis of variance to determine changes in the distribution of FA in the different parts

of the tea shoot. Shoots, i.e. bud, 1st leaf, 2nd leaf, 3rd leaf, 4th leaf, 5th leaf, 6th leaf and stem were the treatments.

Analysis of variance to determine changes in the FA of two clones due to different plucking standards was done using a 4 × 2 factorial program. The plucking standards, buds, one leaf and a bud, two leaves and a bud and three leaves and a bud, were the main treatments, while the clones 6/8 and 31/8 were the sub-treatment. When only trace amounts of C16:1 were detected, the value 0·001 mg/100 g dry weight, was used to do the ANOVA.

RESULTS AND DISCUSSION

Lauric acid (C12:0) and myristic acid (C14:0) were not detected in the samples. Irrespective of the plucking standard or portion of the shoot, the order of occurrence of the FA was linolenic (C18:3) > linoleic acid (C18:2) > palmitic acid (C16:0) > stearic acid (C18:0) > oleic (C18:1) > palmitoleic (C16:1) acid.

Palmitic and stearic acids are saturated FA and do not break down to form volatile flavour compounds (VFC) during tea manufacture. Thus, although the two acids exist in reasonable quantities in the different portions of the shoot, the acids may not contribute to the volatile flavour quality in black tea. The unsaturated FA, however, are the major precursor compounds in the formation of the group I VFC (i.e. VFC which impart inferior quality to tea) (Owuor *et al.*, 1987*a*,*b*; 1988). Linolenic acid dominated the FA composition irrespective of the portion of the shoot or plucking standard. Normally, the unsaturated C_6 aldehydes and alcohols dominate the group I VFC composition (Owuor *et al.*, 1987*b*, 1988; Mahanta *et al.*, 1988). The presence of large amounts of these VFC in black tea is explained by the high quantities of their precursor acid (linolenic acid).

Hexanal and *n*-hexanol produced from linoleic acid are next in dominance of the group I VFC of black tea (Owuor *et al.*, 1988; Mahanta *et al.*, 1988). It is noted in this study that linoleic acid is also next in the dominance of FA composition in green tea leaf. Only low levels of nonanal and *n*-nonanol from oleic acid, heptanal and *n*-heptanol from palmitoleic acid have been detected in Kenyan teas (Horita & Owuor, 1987). The low levels of these precursor acids in tea shoots explain the low occurrence of these VFC in black tea. However, it is noted, that there cannot exist direct linear proportionality in the sum of the VFC to their precursor acids. Volatility of the VFC are different and, during black tea manufacture, more of the more volatile VFC are lost.

The distribution of the FA in the different portions of the tea shoots is presented in Table 1. There were significant changes in all the FA in the

TABLE 1
Distribution of Fatty Acids (FA) (mg/100 g dry wt) in Different Parts of the Tea Shoot

Portion of shoot	*Fatty acids*								
	C16:0	*C16:1*	*C18:0*	*C18:1*	*C18:2*	*C18:3*	*Total FA*	*Total unsaturated FA*	*C18:2 plus C18:3*
Bud	18·68	Trace	10·08	5·58	20·75	53·49	108·6	79·83	74·25
1st leaf	11·52	Trace	11·51	5·53	18·74	62·64	109·9	86·91	81·37
2nd leaf	11·31	0·84	14·36	5·72	18·74	72·83	123·8	98·13	91·57
3rd leaf	10·23	0·81	14·64	4·82	15·90	81·76	128·2	103·3	97·66
4th leaf	10·17	0·84	13·47	3·75	12·38	91·13	131·7	108·1	103·5
5th leaf	11·76	Trace	13·46	3·74	9·26	96·24	134·7	109·5	106·1
6th leaf	11·90	Trace	15·16	3·82	9·29	100·66	140·8	113·8	110·0
stem	6·96	Trace	1·27	1·33	10·16	25·29	45·02	36·78	35·45
CV (%)	8·22	6·64	9·29	14·72	14·06	8·33	6·30	77·41	7·61
LSD									
$P = 0·05$	1·66	0·04	1·91	1·11	3·55	10·65	12·73	11·94	11·66
$P = 0·01$	2·31	0·05	2·65	1·53	4·92	14·79	17·67	16·57	16·19
$P = 0·001$	3·21	0·07	3·69	2·13	6·84	20·56	24·57	23·05	22·51

TABLE 2

Changes in the Fatty Acid (FA) Levels (mg/100 g dry wt) (as fatty acid methyl esters) with Plucking Standards and Clones

Fatty acid	*Clone*	*Plucking standard*				*Mean: clone*		
		Bud	*One and a bud*	*Two and a bud*	*Three and a bud*			
C16:0	6/8	20·17	22·89	16·81	13·74	18·40	CV (%)	= 1·96
	31/8	21·49	18·78	18·10	16·93	18·82	LSD *P*	= 0·05
			Mean: plucking standard				Plucking standard (*a*)	= 0·45
		20·83	20·84	17·46	15·34		clones (*b*)	= 0·32
							a × *b*	= 0·64
C16:1	6/8	Trace	Trace	1·02	0·78	0·45	CV (%)	= 39·17
	31/8	Trace	0·92	0·88	1·31	0·78	LSD *P*	= 0·05
			Mean: plucking standard				Plucking standard (*a*)	= 0·30
		Trace	0·46	0·95	1·05		clones (*b*)	= 0·21
							a × *b*	= 0·42
C18:0	6/8	15·45	14·62	13·85	14·09	14·50	CV (%)	= 6·91
	31/8	14·09	14·61	14·21	14·35	14·32	LSD *P*	= 0·05
			Mean: plucking standard				Plucking standard (*a*)	= NS
		14·77	14·61	14·03	14·22		clones (*b*)	= NS
							a × *b*	= NS
C18:1	6/8	5·33	5·29	7·53	6·33	6·12	CV (%)	= 2·99
	31/8	5·25	8·23	9·32	8·46	7·82	LSD *P*	= 0·05
			Mean: plucking standard				Plucking standard (*a*)	= 0·26
		5·29	6·76	8·43	7·40		clones (*b*)	= 0·18
							a × *b*	= 0·37

C18:2	6/8	35·09	34·23	34·87	35·81	35·00	CV (%)	= 3·01
	31/8	34·03	31·22	31·46	33·12	32·46	LSD *P*	= 0·05
			Mean: plucking standard				Plucking standard (*a*)	= 1·26
		34·56	32·73	33·17	34·47		clones (*b*)	= 2·35
							a × *b*	= NS
C18:3	6/8	116·1	127·6	130·7	134·3	127·22	CV (%)	= 2·14
	31/8	118·0	123·4	125·9	129·06	124·1	LSD *P*	= 0·05
			Mean: plucking standard				Plucking standard (*a*)	= 3·32
		117·1	125·5	128·3	131·75		clones (*b*)	= 2·35
							a × *b*	= NS
Total FA	6/8	193·8	204·7	204·8	205·18	202·1	CV (%)	= 1·59
	31/8	192·9	197·2	199·9	203·22	198·3	LSD *P*	= 0·05
			Mean: plucking standard				Plucking standard (*a*)	= 3·94
		193·4	200·9	202·3	204·2		clones (*b*)	= 3·87
							a × *b*	= NS
Total unsaturated FA	6/8	158·2	167·2	174·1	177·32	169·2	CV (%)	= 1·68
	31/8	157·3	163·8	167·57	171·94	165·1	LSD *P*	= 0·05
			Mean: plucking standard				Plucking standard (*a*)	= 3·48
		157·8	165·5	170·9	174·63		clones (*b*)	= 2·46
							a × *b*	= NS
C18:2 plus C18:3	6/8	152·9	161·9	165·4	170·25	162·6	CV (%)	= 1·72
	31/8	152·0	154·6	157·4	162·19	156·6	LSD *P*	= 0·05
			Mean: plucking standard				Plucking standard (*a*)	= 3·41
		152·5	158·2	161·5	166·22		clones (*b*)	= 2·41
							a × *b*	= NS

NS = not significant.

different parts of the shoot. The stem had the lowest accumulation of FA except linoleic acid which was lower in the 5th and 6th leaf. The bud contained high amounts of palmitic acid. However, there was no difference in the palmitic levels of 1st leaf to 5th leaf. Reasonable quantities of palmitoleic acid were detected in the 2nd to 4th leaves, but the bud, 1st leaf, 5th leaf, 6th leaf and the stem only had trace amounts of the acid. Stearic acid distribution in different portions of the shoots did not follow a pattern while the oleic acid levels and the linoleic acid levels decreased as the leaf matured. The linolenic acid, total FA and total unsaturated FA levels, however, increased with increase in maturity of the leaf. The increase in total unsaturated FA or linolenic plus linoleic acid with maturity of the shoots implies that as the leaves become maturer their tendency to produce high quality teas decreases.

Of the TRFK released clones, clones 6/8 and 31/8 are the most extensively planted by the farmers. Clone 6/8 is widely planted due to the high quality of its black teas while at the same time having reasonable yields, while clone 31/8 is extensively planted because of its high yields and acceptable quality of its black tea. The effects of different plucking standards on the FA levels of the two clonal tea shoots are presented in Table 2. Except for stearic acid, there were significant individual and total FA differences due to the clones. Similar differences in the group I VFC of the two clones had been noted before (Owuor *et al.*, 1988). Thus these two clones indeed are different in their flavour quality nature due to the variations in the precursor FA responsible for biosynthesis of the group I VFC.

Although palmitic and stearic acid levels may not affect the flavour quality of teas, the palmitic acid values significantly increased with coarser plucking standard, while stearic acid levels did not change as plucking standards varied. The levels of all the FA responsible for the production of the VFC increased significantly as the plucking standard became coarse, i.e. as more mature shoots were plucked. This increase was also reflected in the total FA, total unsaturated FA and linolenic plus linoleic acid. Thus as the plucking standard gets coarser, the resultant black teas would have poorer flavour quality due to increase in the group I VFC (Owuor *et al.*, 1987*a*) arising from the increase in the precursor FA noted here.

The decision on the correct plucking standard should therefore take into account quality consideration. A coarse plucking standard would result in quality losses. Whereas, in a plucking round, coarse plucking standards would produce high yields, it has been shown (Odhiambo, 1988) that a fine plucking standard at more frequent intervals produces higher yields than coarse plucking at less frequent intervals. If farmers can reduce their plucking intervals, and pluck fine, there would be a general quality improvement without losses in yields.

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