

The changes in black tea quality due to variations of plucking standard and fermentation time

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Catechin levels in green leaf, total theaflavins, brightness, thearubigins, colour, flavour index and sensory evaluation decrease with coarse plucking standard in clone 6/8. The sum of Group I volatile flavour compounds and C₆ aldehydes and alcohols, in particular, increases with coarse plucking standard. The sum of Group II did not follow a particular pattern, but flavour index decreased with coarse plucking standards. The decrease in total theaflavin levels was due to the decrease in all the individual theaflavins. Although all the individual theaflavin levels decreased with coarse plucking standards, the decrease was greater in galloylated theaflavins, especially in theaflavin digallate, compared with simple theaflavin. Short fermentation times produced black teas with higher brightness and flavour index, but lower theaflavins, thearubigins and colour, irrespective of plucking standard. Total theaflavins reached a maximum after 90 min of fermentation, irrespective of the plucking standard, whereas ungalloylated theaflavin levels decreased with extended fermentation duration at all plucking standards, theaflavin-3-gallate and theaflavin-3,3'-digallate levels increased, resulting in the increase in theaflavin digallate equivalent. Brightness and flavour index decreased while thearubigins, colour, and Groups I and II volatile flavour compound levels increased with long fermentation. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Agronomic/cultural practices affect the quality of black tea from *Camellia sinensis* (L.) O. Kuntze. One such agronomic/cultural practice reported to affect black tea quality is plucking standard. Coarse plucking standard reduces black tea quality (Owuor *et al.*, 1987, 1990; Owuor, 1989, 1990), due to the decline in catechin levels (Forrest and Bendall, 1966) and changes in polyphenol oxidase isoenzyme composition and activity (Takeo and Baker, 1973; Thanaraj and Seshadri, 1990; Obanda and Owuor, 1992), leading to a general decline in total theaflavin levels (Owuor *et al.*, 1987; Owuor, 1990). There is concomitant increase in unsaturated fatty acids (Owuor *et al.*, 1990), leading to production of less aromatic black teas (Owuor *et al.*, 1987).

During black tea processing, fermentation conditions of time, temperature, humidity, etc., have been documented to cause changes in the chemical composition and hence quality of black tea at two leaves and a bud plucking standard (Cloughley, 1979; Owuor and Reeves, 1986). As a result, the conditions, especially fermentation duration, are normally very closely monitored during black tea processing.

Although the recommended plucking standard in Kenya is two leaves and a bud (Othieno, 1988), some farmers are known to pluck coarser leaf. Such a practice is erroneously thought to lead to higher crop volume, even though it sacrifices quality. The proponents of the practice argue that the extra crop, obtained by coarse plucking per plucking round, more than compensates for loss in quality. However, recently it was demonstrated that, provided plucking rounds are shortened, fine plucking standards actually improve yields (Odhiambo, 1989; Owuor and Odhiambo, 1994). Thus, unless labour is limiting, fine plucking can be carried out at shorter intervals, resulting in improved yields (Odhiambo, 1989; Owuor and Odhiambo, 1994) and quality (Owuor *et al.*, 1990).

Despite the knowledge that green leaf catechin levels and composition (Forrest and Bendall, 1966; Obanda and Owuor, 1992) and/or polyphenol oxidase activity (Takeo and Baker, 1973; Thanaraj and Seshadri, 1990; Obanda and Owuor, 1992) decline with coarse plucking standards, black tea manufacturers normally set the same fermentation time, with changes only being instituted to take into account the variations in ambient temperatures. Thus fermentation times are shortened

during hot conditions and lengthened during cold conditions. Variations do not normally take into account the standard of plucking of the leaf. This study was done to determine whether fermentation time should be varied when the plucking standard is changed so that black tea of optimum quality is obtained.

MATERIALS AND METHODS

Leaf for manufacture was obtained from fields of clone 6/8 grown on Timbilil Estate, Tea Research Foundation of Kenya (TRFK), altitude 2180m a.m.s.l, and receiving 150 kg N ha⁻¹year⁻¹ as NPKS 25:5:5:5 compound fertilizer in a single dose 4 months prior to the experiment. The plants were allowed to over-grow so that it was possible to pluck up to four leaves and a bud. The plucked leaf was then sorted into different plucking standards as follows: one leaf and a bud (1 + bud), two leaves and a bud (2 + bud), three leaves and a bud (3 + bud), and four leaves and a bud (4 + bud), all inclusive of the stems.

After sorting, 100 g of leaf from each plucking standard was steamed for 2 min and then dried in an oven at 80°C for 6 h. The dried leaf was ground to powder, of which (0.2 g) was extracted with 50 ml of 80% aqueous acetone in a water bath at 45°C for 30 min. The extract was decanted and filtered through a millipore film (0.45 µm), and dried in a rotary evaporator at 40°C. The residue was dissolved in 20 ml distilled water, which was extracted four times with 20 ml chloroform. The pH of the water layer was reduced to 2 using 1 M HCl. The solution was extracted with isobutyl methyl ketone (IBMK) (20 ml × 2). The IBMK was removed using a rotary evaporator and the residue dissolved in 10 ml 2% aqueous acetic acid. One millilitre of the solution was eluted through a C₁₈ cartridge with 5 ml 12% acetonitrile and 2% acetic acid in water and the eluted catechins analyzed (Obanda and Owuor, 1992).

Each plucking standard was done in triplicate in 1200 g batches. The leaf was placed on open miniature withering troughs for 16–18 h to achieve 69–70% wither and subsequently macerated in a miniature CTC machine. Every plucking standard was then fermented for 60, 90 and 120 min at 22–26°C. The fermented 'dhoor' was fired in a miniature fluid bed drier to a residual moisture content of 3–5%.

The unsorted black tea was subjected to chemical analysis and sensory evaluation. The total theaflavins

and brightness were assayed by the Flavognost method (Hilton, 1974), while the thearubigins and colour measurements were done as outlined by Roberts and Smith, (1963). Caffeine was quantified by the method of Cloughley, (1982). Individual theaflavins were determined by HPLC (Obanda and Owuor, 1992).

The volatile flavour compounds (VFC) were extracted by the method of Likens and Nickerson, (1964) as modified by Baruah *et al.*, (1986), using cumene as the internal standard (15 µg per gram of dry black tea). The VFC were analyzed under the conditions of Baruah *et al.*, (1986) and the gas chromatographic peaks quantified by area relative to that of the internal standard (Baruah *et al.*, 1986; Owuor *et al.*, 1986, 1987, 1990). The VFC were grouped into those imparting inferior aroma (Group I VFC) and those imparting sweet flowery aroma (Group II VFC), as explained earlier (Owuor *et al.*, 1986, 1987, 1990; Owuor, 1992). The ratio of Group II to Group I (Owuor's flavour index (FI)) can be used to qualitatively classify Kenyan black tea in terms of aroma quality (Owuor *et al.*, 1986, 1987, 1988, 1990; Owuor, 1992).

Sensory evaluation was carried out by two panels of professional black tea tasters and scores were based on briskness, brightness, infusion, colour, thickness, flavour and overall quality on a scale of 0 to 10 for panel A, and 0 to 20 for panel B for each component. The data were subjected to analysis of variance using factorial two in randomized complete block arrangement. But for the individual theaflavins analysis, samples from different replicates were bulked and analyses done in duplicate.

RESULTS AND DISCUSSION

The changes in the individual catechin levels with level of plucking standards are summarised in Table 1. There was a general decline in the catechin levels with coarseness of plucking standard, which is similar to that observed by Forrest and Bendall, (1966). The ratios of the different catechins also changed with plucking standard. Thus, although epigallocatechin gallate was the most dominant and (+)-catechin the least dominant flavanol, the rate of decline in the individual catechins with change in plucking standard was not uniform. (+)-Catechin showed the least variation, while epigallocatechin gallate and epigallocatechin had the highest

Table 1. Changes in the catechins (mg g⁻¹) and caffeine levels (%) with plucking standards

Plucking standard	EGC	+C	EGCG	EC	ECG	Caffeine
1 + bud	3.94(-) ^a	2.00(-)	25.0	13.3	9.31(-)	2.40
2 + bud	3.00(-23.9)	1.18(-41.0)	19.0(-23.4)	12.3(-10.2)	9.26(-0.5)	2.28
3 + bud	2.96(-24.9)	2.47(+23.5)	17.0(-32.8)	10.9(-20.5)	7.97(-14.4)	2.06
4 + bud	1.35(-65.7)	1.88(-6.0)	13.0(-48.4)	8.28(-39.5)	6.64(-28.7)	1.69

^aNumbers in parentheses are percent changes relative to one leaf and a bud. EGC = Epigallocatechin, +C = (+)-Catechin, EGCG = Epigallocatechin gallate, EC = Epicatechin, ECG = Epicatechin gallate.

variations due to plucking standards. Polyphenol oxidase activity had also been demonstrated to decline with coarse plucking standard (Takeo and Baker 1973; Thanaraj and Seshadri, 1990). In the polyphenol oxidase-catalysed reaction, one molecule of a simple catechin reacts with a gallocatechin to form a theaflavin molecule (see Fig. 1). The catechins differ in their redox potentials causing variations in the rate of their decline during black tea fermentation. The decline in catechin levels and polyphenol oxidase activity, with coarse plucking standard, resulted in declines in both the thearubigins, total theaflavin levels and the level and composition of the individual theaflavins (Tables 2 and 3). Generally, brightness levels and colour of black tea are attributed to theaflavins and thearubigins, respectively. The changes in brightness and colour with variation in plucking standard paralleled the changes in theaflavins and thearubigins, respectively (Table 2). However, the changes in the composition of the catechins with plucking

standard resulted in the changes in the total theaflavin and individual theaflavins levels and ratios with plucking standards and fermentation durations (Table 3).

In the fermentation temperature range of 22–26°C, 90 min fermentation is normally ideal for Clone 6/8 (Owuor, unpublished) for production of good quality black tea at two leaves and a bud plucking standard (Owuor and Reeves, 1986). Generally, it is thought that the optimum fermentation time for black tea manufacture is when maximum theaflavins are formed (Cloughley, 1979; Owuor and Reeves, 1986). In this study, this occurred after about 90 min (Table 2). The range 60 to 120 min used for this study falls within the period when theaflavin formation reaches a plateau (Owuor and Reeves, 1986) and in fact the differences in total theaflavin levels due to variation of fermentation time between 60 and 120 min were not significant. Brightness significantly ($p < 0.01$) declined with lengthening of fermentation, while colour and thearubigin levels increased

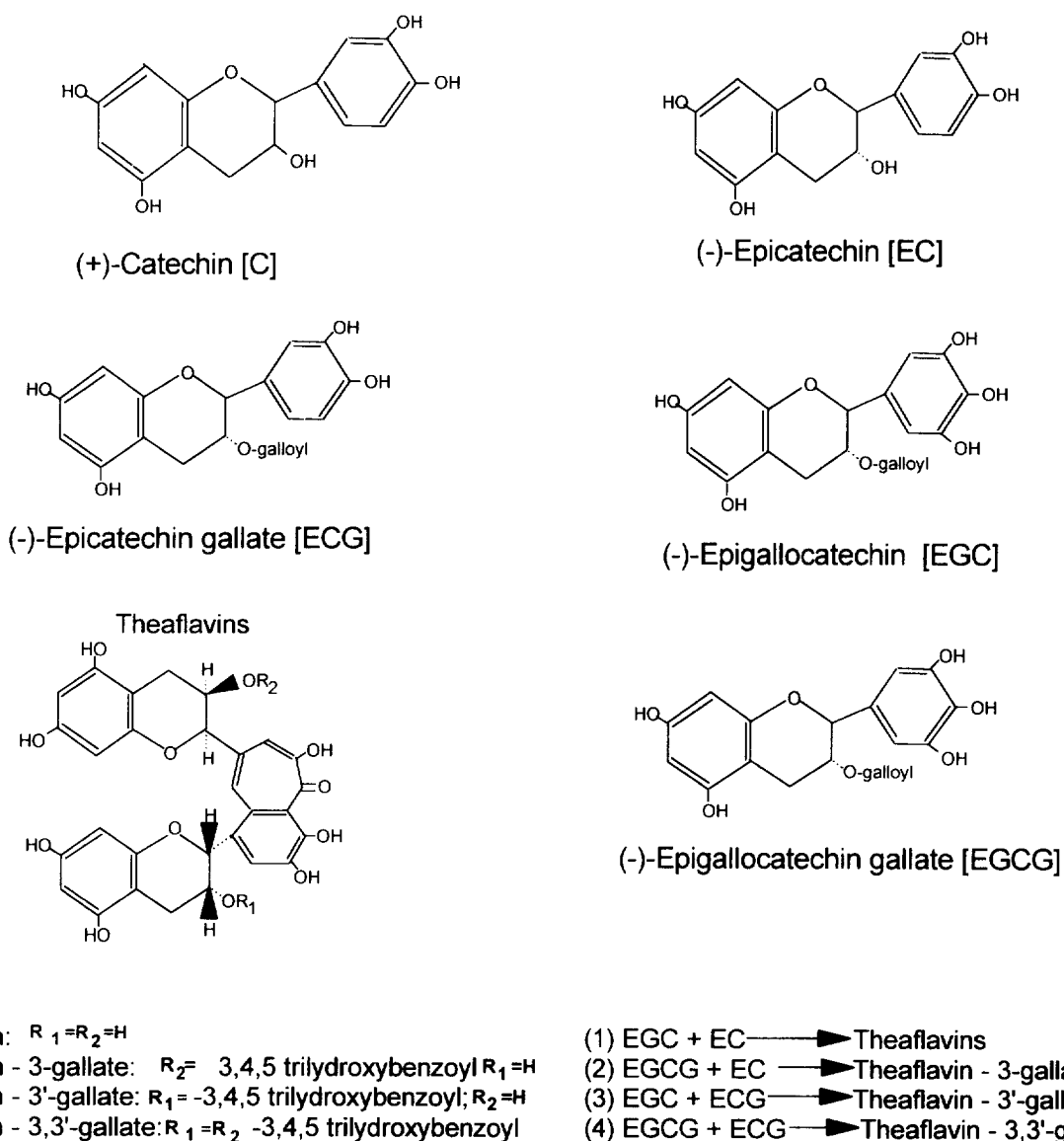


Fig. 1. Structures and general scheme for the formation of individual theaflavins.

Table 2. Changes in black tea plain tea quality parameters due to plucking standard and fermentation time

(a) Theaflavins ($\mu\text{moles g}^{-1}$)					
Fermentation time (min)	Plucking standard				Mean
	1 + bud	2 + bud	3 + bud	4 + bud	
60	24.7	24.0	21.2	17.3	21.8
90	24.5	24.8	20.4	19.20	22.2
120	24.9	23.1	21.2	18.6	22.0
Mean	24.7	24.0	20.9	18.4	
C.V.%		9.08			
LSD ($p < 0.05$)		1.95			NS
(b) Brightness (%)					
Fermentation time	1 + bud	2 + bud	3 + bud	4 + bud	Mean
60	34.09	34.03	34.58	29.67	32.99
90	30.65	31.41	30.88	27.60	30.14
120	29.34	28.45	26.37	23.11	26.82
Mean	31.36	31.30	30.61	26.66	
C.V.%		8.64			
LSD ($p < 0.05$)		2.53			2.19
(c) Thearubigins (%)					
Fermentation time	1 + bud	2 + bud	3 + bud	4 + bud	Mean
60	11.9	13.0	12.2	12.1	12.3
90	13.7	13.8	12.9	13.0	13.4
120	14.3	14.9	14.4	14.0	14.4
Mean	13.3	13.9	13.1	13.0	
C.V.%		6.00			
LSD ($p < 0.05$)		0.78			0.68
(d) Colour (%)					
Fermentation time	1 + bud	2 + bud	3 + bud	4 + bud	Mean
60	4.87	4.53	4.02	3.31	4.18
90	5.45	4.99	4.22	4.00	4.66
120	5.79	5.36	4.69	4.35	5.05
Mean	5.37	4.96	4.31	3.89	
C.V.%		6.19			
LSD ($p < 0.05$)		0.28			0.24

($p < 0.001$) with long fermentation times. Although it had been thought that there could be plucking standard-fermentation time interactions, no significant effects were recorded. Caffeine levels also declined with coarseness of plucking standard (Table 1). Thus, despite the decline in polyphenol oxidase activity (Takeo and

Baker, 1973; Thanaraj and Seshadri, 1990), and catechin levels and/or change in catechin composition (Forrest and Bendall, 1966; Obanda and Owuor, 1992), it seems that for the manufacture of plain teas, the same fermentation time can be used irrespective of plucking standards. The variations in plain black tea quality

Table 3. Changes^a in the total theaflavins, individual theaflavins and theaflavin digallate equivalent levels with plucking standards and fermentation durations

Plucking standard	Fermentation time	Total (Flavognost) Theaflavins	Theaflavin	Theaflavin -3-gallate	Theaflavin -3'-gallate	Theaflavin -3,3'-digallate	Theaflavin -digallate equivalent
1 + bud	60	25.7	15.6	4.74	3.47	1.92	7.20
	90	27.7	15.3	6.25	3.58	2.53	8.34
	120	28.5	15.0	7.23	3.43	2.82	8.87
	Mean	27.3(-) ^b	15.3(-)	6.07(-)	3.49(-)	2.42(-)	8.14(-)
2 + bud	60	22.0	14.7	3.70	2.56	1.07	5.53
	90	24.4	14.5	5.09	3.87	0.96	6.33
	120	24.45	14.56	5.56	2.78	1.53	6.7
	Mean	23.6(-13.5)	14.6(-4.8)	4.78(-21.3)	3.07(-12.0)	1.19(-50.8)	6.19(-24.0)
3 + bud	60	22	14.7	3.65	2.5	1.10	5.54
	90	23.3	15.8	4.26	2.1	1.12	5.79
	120	22.34	14.2	4.52	2.22	1.40	5.96
	Mean	22.5(-17.5)	14.9(-2.7)	4.14(-31.8)	2.27(-35.0)	1.21(-50.0)	5.76(-29.2)
4 + bud	60	18.9	14.5	2.03	1.68	0.75	4.29
	90	22.8	15.1	4.62	1.99	1.15	5.79
	120	22.8	14.7	4.88	1.98	1.18	5.85
	Mean	21.5(-21.3)	14.7(-3.8)	3.84(-36.7)	1.88(-46.1)	1.03(-57.4)	5.31(-34.8)

^a Results given are means of two analyses of bulked samples from various replicates. The analyses varied by less than 5%.

^b Numbers in parentheses are percent changes relative to one leaf and a bud.

Table 5. Changes in black tea aroma parameters due to variation in plucking standard and fermentation time (scopes of overall sensory acceptability out of 10)

(a) Group I VFC		1 + bud	2 + bud	3 + bud	4 + bud	Mean
Fermentation time (mins)						
60		1.83	1.90	3.17	3.20	2.53
90		1.95	2.50	3.50	3.16	2.78
120		2.04	3.16	3.57	3.22	3.00
Mean		1.94	2.52	3.41	3.19	
C.V.%			10.66			
LSD($p < 0.5$)			0.29			0.25
(b) Group II VFC						
Fermentation time (min)						
60		3.82	3.70	4.17	2.48	3.56
90		3.52	3.65	4.41	3.10	3.68
120		3.79	4.67	3.22	3.43	3.78
Mean		3.74	4.01	3.93	3.01	
C.V.%			12.68			
LSD($p < 0.5$)			0.74			NS
(c) FI						
Fermentation time (min)						
60		2.12	2.05	1.28	0.78	1.56
90		1.82	1.51	1.25	1.01	1.40
120		1.84	1.47	0.91	1.07	1.32
Mean		1.92	1.68	1.15	0.95	
C.V.%			13.37			
LSD($p < 0.5$)			0.33			NS

was a general increase in the total sum of Group I and decrease in Group II VFC levels with coarse plucking standards resulting in decline in FI. These results are similar to previously published data (Owuor *et al.*, 1987). The changes in black tea aroma with fermentation time are not properly documented. Both the Group I and Group II VFC increased with increase in fermentation time. However, the rate of increase in the Group I VFC levels was faster than that of Group II VFC resulting in a general decline in the aroma quality as measured by FI. Thus measured by both plain and aroma tea quality parameters, shorter fermentation times make superior black teas irrespective of plucking standard.

Although the use of sensory evaluation is always criticised as being subjective and influenced by market factors such as demand, supply and consumer preferences (Biswas *et al.*, 1971) and personal preferences of the individual tasters, it still remains the fastest and the most practical method of quality assessment in the black tea trade. The changes in sensory evaluation due to variations in plucking standards and fermentation times are presented in Table 5. As with both plain tea quality parameters and black tea aroma, tasters preferred finely plucked leaf and shorter fermentation times. There were no significant interaction effects between plucking standard and fermentation times. The results presented here confirm the factory practice where fermentation times are not changed with changes in plucking standard. Thus, despite the decrease in the

catechin levels and polyphenol oxidase activity in green tea leaves due to plucking standards, there are no effects of plucking on the optimal fermentation duration.

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