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Catechins depletion patterns in relation to theaflavin and thearubigins formation

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ABSTRACT

The determination of the depletion pattern of catechins in black tea processing is important in achieving optimum tea quality. This study investigated catechins (unoxidised di- and trihydroxylated) depletion patterns in relation to theaflavin and thearubigin formation. It was during the process of green leaf fermentation at selected temperature and time combinations. The tea leaves were obtained from three clones (6/8, 303/577 and 311/287) within the Tea Research Foundation of Kenya. The results were showed that unequal depletion rates of di- and trihydroxylated catechins led to a decline in total theaflavin and an increase in thearubigins levels. An equitable decline in both groups of catechins corresponded to a subsequent rise in theaflavins content. The decline in the catechins levels was much faster at higher temperatures resulting in a shorter fermentation time to achieve a peak of the theaflavins content. Clone 311/287 had the highest mean theaflavins content (26.99 μmol/g) and the least mean percent thearubigins (15.02%) level. Theaflavins content correlated positively with liquor brightness determined by a spectrophotometer and tea tasters ($r = 0.7221$, $p < .0001$). The thearubigins content was however found to relate negatively with liquor brightness. It was concluded that the experimental conditions tested form a good basis for clonal specific processing conditions that can be utilised in manufacturing quality black tea.

1. Introduction

Commercial production of tea beverages from *Camellia sinensis* (L.), O. Kuntze is widely distributed in the world. The green leaf can be processed into green tea (non fermented type), oolong (semi fermented tea) or black tea (naturally oxidised tea or microbial fermented tea). Approximately 76–78% of the tea produced and consumed worldwide is black tea (Cabrera et al., 2003). During the black tea manufacture, a natural enzyme catalyses oxidation and condensation of green leaf tea catechins leading to the formation of theaflavins (TF) and thearubigins (TR) (Robertson, 1992). This process is normally referred to as fermentation even if no microorganisms are used in this kind of enzyme-oxidized black tea (Mo, Zhu, & Chen, 2008).

Most of Kenyan black teas are classified as plain to medium flavour in the international market (Owuor, 1996). The plain black teas are evaluated on the basis of their briskness, brightness, strength, body and total colour of liquors (Roberts & Smith, 1963). These attributes of black tea quality are mainly dictated by the levels of theaflavins and thearubigins (Robertson, 1992). Theaflavins are responsible for the astringency, brightness, colour and briskness of the black tea. Thearubigins contribute to the mouth feel (thickness) and colour of the tea (Biswas, Biswas, & Sarkar, 1973). Black tea quality is mainly influenced by total theaflavins (Wright, Mphangwe, Nyirenda, & Apostolides, 2002) or derived theaflavin digallate equivalents (Owuor & Obanda, 1997). The structures of the catechins and theaflavins are shown in Figs. 1 and 2, respectively.

The formation of a single theaflavin requires a dihydroxy and trihydroxy flavan-3-ols. The ratio of dihydroxyflavan-3-ol to trihydroxy-3-ol in green leaf may thus have a major influence on the amount of theaflavins in black tea. The correct balance and amount of dihydroxyflavan-3-ol and trihydroxyflavan-3-ol are therefore necessary to ensure maximum formation of the theaflavins (Wright et al., 2002). The amount of the individual theaflavins are formed largely influenced by the amounts of the precursor catechins in green leaf, their redox potential and/or affinity for polyphenol oxidase and activity (Owuor & Obanda, 2007).

Fermentation is a critical stage in the manufacture of black tea during which oxidative condensation of catechins to TF and TR occurs. Catechins, together with their oxidation products are responsible for most of the sensory characteristics associated with black tea liquors (Biswas et al., 1973). Temperature and time are important factors in determining the extent of fermentation. Processing conditions which favour less degradation of simple theaflavins and the retention of higher epicatechin gallate (ECG) and epigallocatechin gallate (EGCg) levels produce more brisk tea liquors (Obanda, Owuor, & Mang'oka, 2001). Theaflavins and unoxidized

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catechins are thought to have considerable human health benefits (Apostolides & Weisberger, 1995). Therefore, tea clones or processing conditions with a high potential of achieving this, can enhance the health benefit potential of black tea and impact positively on liquor astringency.

Significant interactions between fermentation duration and temperatures and all plain black tea quality parameters have been reported (Owuor & Obanda, 2001). In a previous study by Obanda, Owuor, and Mang'oka (2001), theaflavins formed over time was observed to be dependent on temperature. It is therefore important to establish optimal fermentation temperature and duration for clones that are commercially grown, for production of high quality black teas.

This study was done to determine catechins depletion patterns in relation to theaflavins and thearubigins formation at different fermentation temperatures and duration.

2. Materials and methods

2.1. Tea manufacture

Green leaf (two leaves and a bud) was obtained from the fields at the Tea Research Foundation of Kenya (TRFK). The clones 6/8, 303/577 and 311/287 were sampled from the Timbilil Estate of TRFK at an altitude of 2178 m a.m.s.l and latitude 0°22″S. TRFK clone 6/8 is a high quality clone and it is considered a standard in the manufacture of black tea in Kenya. TRFK clone 303/577 is an open pollinated progeny of clone 6/8. It is a high yielder with medium quality and is increasingly being cultivated commercially. TRFK clone 311/287 is a catechin rich tetraploid clone, rich in EGCg, with a pharmacological potential for use in herbal formulations.

Green leaf (12 kg) were plucked from each clone and withered under ambient conditions for 18–22 h. The leaf was 'crushed, torn and curled' (CTC) – macerated and fermented at 18, 24 and 30 °C (wet bulb and dry bulb temperature) for 60, 90, 120 and 150 min in environmentally controlled cabinets (Tea Craft, UK). The fermentation was stopped by drying the 'dhool' to a moisture content of about 3% using a miniature drier (Tea Craft, UK) set at 120 °C. Each treatment was replicated three times.

2.2. Reagents

Authentic flavanol standards ((+)-catechin, C; (−)-epicatechin EC; (−)-epigallocatechin EGC; (−)-epicatechin gallate ECg and (−)-eipigallocatechin gallate (EGCg) were obtained from Sigma chemicals. Isobutyl methyl ketone (IBMK) and Flavognost reagent were purchased from Aldrich Chemicals. All the solvents used were HPLC grade, whilst water was double distilled.

2.3. HPLC analysis of catechins in black tea

HPLC analysis of catechins in the processed black tea under the various fermentation conditions was done by the modified method of Wang, Helliwell, and You (2000) using isocratic elution system. This analysis was done by using a Shimadzu SCL-10A liquid chromatograph equipped with a pump, LC-10AS, thermostatically controlled compartment and a Shimadzu UV–VIS detector (SPD-10AV), and a C-R7A CHROMATOPAC data processor (Kyoto, Japan). The tea samples were prepared according to the conventional tea brewing method. The samples (3 g) were infused with 150 ml of boiling deionized distilled water for 5 min. The infusions were then filtered and cooled to room temperature and then filtered through a 0.45 μm fibre glass filter before HPLC analyses.

Authentic catechins standards of (+)-Catechin, C; (−)-epicatechin, EC; (−)-epigallocatechin, EGC; (−)-epigallocatechin gallate, EGCg; (−)-epicatechin gallate, ECg and gallic acid were use to prepare mixed standards of known concentrations selected to cover the range of compositions typically found in tea.

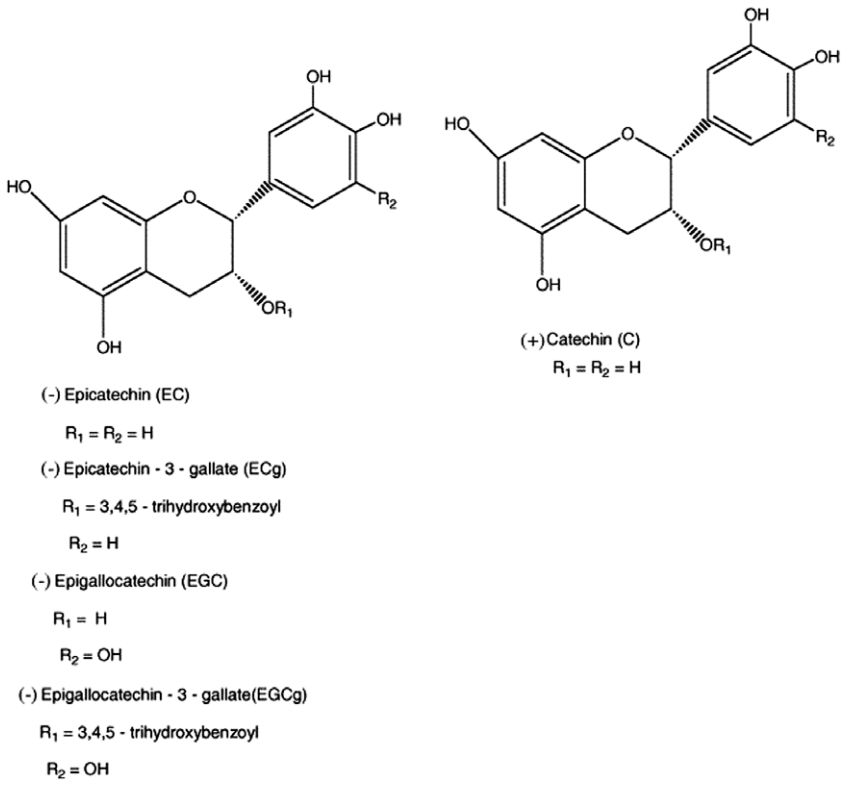
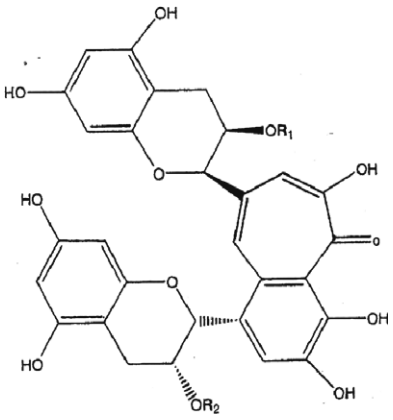


Fig. 1. The flavan-3-ols (catechins) in fresh tea leaves.



I Simple theaflavin (TF)
$R_1 = R_2 = H$
II Theaflavin-3-gallate (TF-3-g)
R_1 = 3,4,5-Trihydroxybenzoyl
$R_2 = H$
III Theaflavin-3'-gallate (TF-3'-g)
$R_1 = H$
R_2 = 3,4,5-Trihydroxybenzoyl
IV Theaflavin-3,3'-digallate (TF-3,3'-dg)
$R_1 = R_2$ = 3,4,5-Trihydroxybenzoyl

Fig. 2. The major individual theaflavins in black tea.

2.4. Determination of plain black tea quality parameters

2.4.1. Determination of dry matter content

Black tea (5 g) of were weighed to the nearest 0.001 g, placed in aluminium dishes and heated in an oven at 103 ± 2 °C for at least 16 h to constant weight. The percentage of dry matter (DM) in the sample was then calculated.

2.4.2. Total theaflavins content analysis (Flavognost)

Total theaflavins content was determined by the Flavognost method as described by Hilton (1973). In brief, a tea infusion was made by adding 375 ml of boiling distilled water into a flask containing 9 g of tea. The flask was shaken for 10 min and the infusion was filtered through a rough cotton wool after which it was allowed to cool at room temperature. The infusions (10 ml) were pipette into 10 ml of isobutylmethylketone; (4-methylpentan-2-one, IBMK). The mixture was shaken for 10 min and allowed to stand until the layers separated. The upper layers (2 ml) were pipette into a test tube followed by 4 ml of ethanol and 2 ml of Flavognost reagent (2 g diphenylboric acid-2-aminoethyl ester dissolved in 100 ml ethanol). The contents were mixed and allowed to stand for 15 min for the colour to develop. The absorbance (A) at 625 nm was read against an IBMK/ethanol (1:1) blank (Obanda, Owuor, & Mang'oka, 2001)

$$\text{Theaflavin}\,(\mu\text{mol/g}) = A_{625nm} \times 47.9 \times 100/\text{DM}; \quad (1)$$

where 47.9 is a conversion factor attributed to the dilution effect in theaflavin analysis.

2.4.3. Determination of liquor total colour

Filtered standard tea infusion (5 ml) from TF analysis was pipetted into 45 ml of distilled water in a 100 ml conical flask. The solution was shaken well to ensure thorough mixing. The absorbance of this solution at 460 nm was read against distilled water blank. The result was corrected for dry matter content of the black tea samples.

$$\text{Liquor colour} = (A_{460nm} \times 10)/(\text{DM}/100) \quad (2)$$

2.4.4. Spectrophotometric measurements of total thearubigins

The method of Roberts and Smith (1963) was used to determine total thearubigins. Four solutions were made as follows:

Fifty ml of the cool, well shaken and filtered standard tea infusion from theaflavin analysis was mixed with 50 ml IBMK and gently shaken to avoid formation of an emulsion. The layers were allowed to separate and a 4 ml portion of the IBMK layer was taken and made up to 25 ml with methanol in a volumetric flask (solution A). Two ml portions of the aqueous layer was diluted to 10 ml with distilled water and then to 25 ml with methanol (solution B).

Twenty five ml of the remaining initial IBMK layer was mixed with 25 ml of 2.5% aqueous sodium hydrogen carbonate. The mixture was shaken vigorously and the layers allowed to separate. The aqueous layer was then discarded. A 4 ml portion of the washed IBMK layer was made up to 25 ml with methanol (solution C). Two ml of a saturated oxalic acid aqueous solution and 6 ml of water was added to a 2 ml portion of the aqueous layer left from the first extraction with IBMK, and diluted to 25 ml with methanol (solution D).

The absorbance A_A, A_B, A_C and A_D of solutions A, B, C and D were read at 380 and 460 nm using a Cecil Digital Grating spechtrophotometer with distilled water as a blank.

The levels of thearubigins in black tea liquor were calculated according to the method described by Obanda et al. (2001). At 380 nm

$$\%\text{TR}\,(\text{Total}) = (375 \times 0.02 \times 6.25[A_D + A_A - A_C])/(0.733 \times 9 \times \text{DM}/100) \quad (3)$$

2.4.5. Determination of liquor brightness

This was determined from absorbance A_A, A_B and A_C of solutions A, B and C prepared above, and read at 460 nm

$$\text{Brightness}\,(\%) = (100 \times A_C)/(A_A + 2A_B) \quad (4)$$

(Obanda et al., 2001).

2.5. Sensory evaluation

Approximately 2 g of each of the randomly numbered black tea samples were infused in 10 ml for 15 min and subjected to organoleptic evaluation by professional tea tasters from tea broking firms in Mombasa, Kenya. The tea infusions were ranked for liquor briskness and brightness. Liquor brightness was ranked by assessing the appearance of the meniscus formed where the liquor touches the porcelain bowl (Werkhoven, 1974). A scale of 0 to 10 was used to rank each attribute (10, very good; 5, average and 1, poor).

2.6. Statistical analysis

Data analysis was done by using Statistical Analysis Software (SAS, 1995) version 9.1.3. Univariate analysis of variance and general linear model procedure were carried out to determine the effect of clonal variation, fermentation temperature and duration on the black tea quality parameters. Pair wise comparison based on t-test (pdiff) was used for mean separation. Regression analysis was done for various models postulated to determine the relationship between theaflavins and/or thearubigins with liquor brightness. Correlation analysis was also carried out to determine the strength of the relationships established.

3. Results

3.1. Effect of clonal differences on black tea quality parameters and tasters' scores

Clone 6/8 black tea was not significantly ($p > 0.05$) different from clone 303/577 in the levels of theaflavin (μmol/g) and liquor total colour. However, black tea from the two clones were significantly ($p < 0.05$) different in total theaflavins and total colour from clone 311/287. From Table 1, it was observed that the three clones produced tea which differed significantly ($p < 0.05$) in the levels of thearubigins and liquor brightness.

Clone 311/287 black tea had the highest levels of theaflavins, liquor colour and brightness with a mean of 26.99 ± 0.53 μmol/g, 5.71 ± 0.09% and 31.18 ± 0.86%, respectively (Table 1). Clone 303/577 produced tea with the highest mean thearubigin content, 21.05 ± 0.29%.

3.2. Effect of fermentation temperature and duration on black tea quality parameters and tasters' scores

From Table 2a it was observed that black tea fermented at 24 and 30 °C from clone 6/8 did not differ significantly ($p < 0.05$) in percent thearubigins and liquor colour. There was also no difference in mean theaflavins content for clone 303/577 fermented at 18 and 24 °C. Similarly tea fermented at 24 and 30 °C did not differ in total liquor colour (Table 2b). Fermentation at 18 °C yielded black tea with the highest mean theaflavin and liquor brightness for clone 6/8 and 311/287 (Tables 2a and c). However, the highest mean theaflavins content was realised by fermentation at 24 °C for clone 303/577. This peak in theaflavins content was reached after 90 min for all the three clones (Tables 3a–c).

The interaction effect of fermentation temperature and duration had a significant effect on all the black tea quality parameters for clone 6/8 and 303/577 ($p < 0.05$). Temperature-time effect also had a significant ($p < 0.05$) influence on theaflavins, thearubigins and liquor brightness but less significant on colour ($p = 0.0457$) with clone 311/287 black tea.

3.3. Depletion patterns of di- and trihydroxylated catechins in relation to theaflavin and thearubigins formation

There was *a* decline in di- (DI) and trihydroxylated (TRI) catechins at 18 °C for clone 6/8 (Fig. 3). The proportional decline in the two groups of catechins between 60 and 90 min corresponded to the steady increase in theaflavins (TF) (Fig. 3). Trihydroxylated catechins concentration was lower than dihydroxylated catechins but the latter showed a sharp decline between 60 and 90 min of fermentation at 24 and 30 °C for clone 6/8. The different rates of depletion of the catechins led to a decline in total theaflavins. The thearubigins (TR) content increased gradually with fermentation time at all the temperature levels.

Fermentation of clone 303/577 at 18 °C showed a decline for both groups of catechins between 60 and 90 min. This corresponded to the rise in theaflavins content for the same interval (Fig. 4). However, trihydroxylated catechins decreased to non-detectable levels beyond 120 min of fermentation. This caused the theaflavins and thearubigins content to reach a peak. At 24 °C

Table 1
Clonal variation in quality paramaters.

Clone	TF (μmol/g)	TR %	TC	BR
6/8	20.81 b	17.98 b	5.34 b	23.54 b
303/577	20.28 b	21.05 a	5.31 b	17.79 c
311/287	26.99 a	15.02 c	5.71 a	31.18 a
CV	14.04	9.97	10.38	21.43

Means followed by the same letter are not significantly different at $p < 0.05$, $n = 36$.

Table 2a
Variation in clone 6/8 black tea quality parameters with fermentation temperature.

Temp	TF (μmol/g)	TR %	TC	BR
18	22.82 a	16.47 b	5.00 b	29.43 a
24	21.30 b	18.43 a	5.45 a	21.55 b
30	18.31 c	19.03 a	5.56 a	19.65 c
CV	7.47	4.53	4.40	5.83

Means followed by the same letter are not significantly different at $p < 0.05$, $n = 12$.

Table 2b
Variation in clone 303/577 black tea quality parameters with fermentation temperature.

Temp	TF (μmol/g)	TR %	TC	BR
18	21.24 a	20.20 c	5.15 b	22.10 a
24	21.44 a	21.06 b	5.35 a	17.78 b
30	18.15 b	21.89 a	5.42 a	13.49 c
CV	4.78	4.36	2.43	3.65

Table 2c
Variation in clone 311/287 black tea quality parameters with fermentation temperature.

Temp	TF (μmol/g)	TR %	TC	BR
18	29.13 a	13.90 c	5.47 b	35.41 a
24	27.05 b	14.56 b	5.63 b	31.70 b
30	24.81 c	16.61 a	6.02 a	26.43 c
CV	6.09	3.15	6.09	2.76

Means followed by the same letter are not significantly different at $p < 0.05$. $n = 12$.

Table 3a
Variation in clone 6/8 black tea quality parameters with fermentation duration.

Duration	TF (μmol/g)	% TR	BR %	TC
60	19.93 b	16.57 b	27.82 a	4.75 b
90	22.02 a	18.23 a	26.11 b	5.49 a
120	20.69 a b	18.35 a	20.55 c	5.57 a
150	20.61 a b	18.76 a	9.70 c	5.53 a
CV	7.47	4.53	4.40	5.83

Table 3b
Variation in clone 303/577 black tea quality parameters with fermentation duration.

Duration	TF (μmol/g)	% TR	BR %	TC
60	21.15 a	19.62 c	21.41 a	4.77 b
90	21.60 a	20.78 b	19.81 b	5.49 a
120	19.79 b	21.72 a	16.00 c	5.48 a
150	18.56 c	22.08 a	13.94 d	5.51 a
CV	4.78	4.36	2.43	3.65

Table 3c
Variation in clone 311/287 black tea quality parameters with fermentation duration.

Duration	TF (μmol/g)	% TR	BR %	TC
60	26.82 b	13.12 d	35.14 a	5.00 b
90	28.72 a	14.59 c	32.08 b	5.83 a
120	26.38 b	16.59 a	28.21 d	6.16 a
150	26.06 b	15.78 b	29.29 c	5.84 a
CV	6.09	3.15	6.09	2.76

Means followed by the same letter are not significantly different at $p < 0.05$ ($n = 9$).

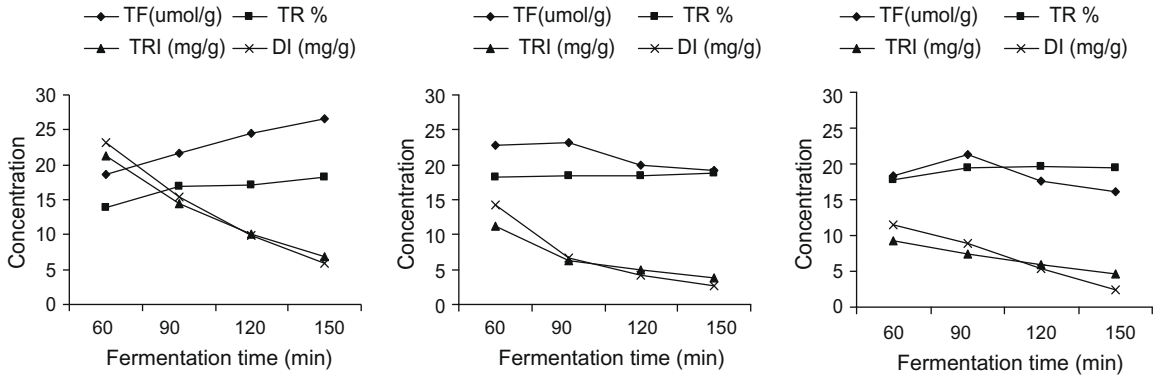


Fig. 3. Di- and trihydroxylated catechins depletion patterns at 18, 24 and 30 °C for clone 6/8.

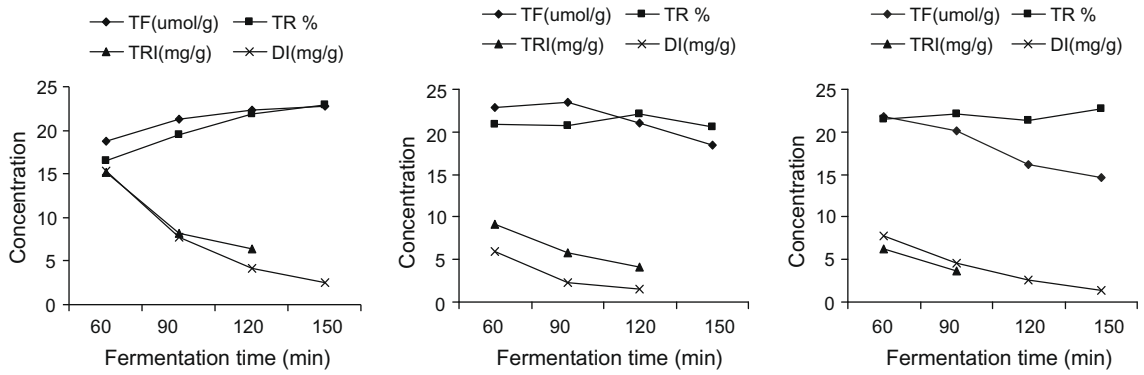


Fig. 4. Di- and trihydroxylated catechins depletion patterns at 18, 24 and 30 °C for clone 303/577.

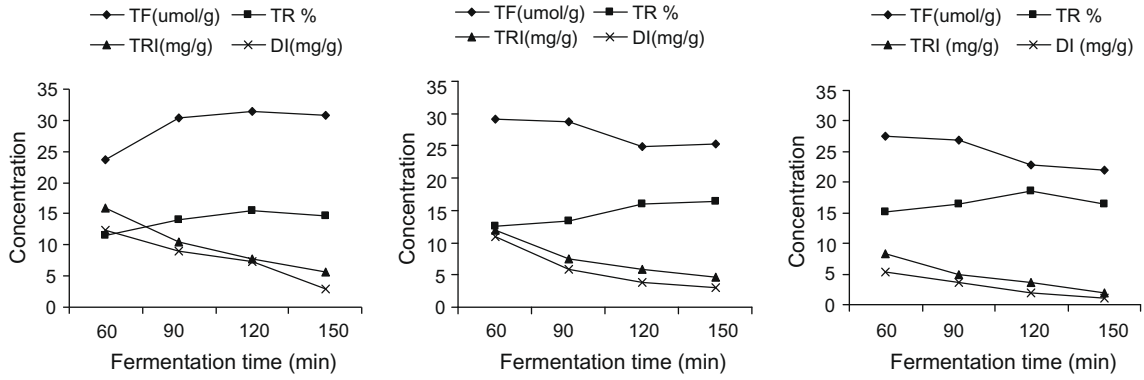


Fig. 5. Di- and trihydroxylated catechins depletion patterns at 18, 24 and 30 °C for clone 311/287.

both groups of catechins were depleted at almost uniform rates between 60 and 90 min of fermentation. This was accompanied by a slight increase in theaflavins and a shorter duration to reach peak concentration. Fermentation at 30 °C led to a faster depletion of trihydroxylated catechins and thus the decline of theaflavins content after 60 min. There was a marked increase in percent thearubigins with decline in theaflavins at 24 and 30 °C.

Fermentation of clone 311/287 resulted in higher levels of total theaflavins but relatively low% thearubigins compared to clone 6/8 and 303/577. Di- and trihydroxylated catechins decreased non-uniformly for 120 min followed by a rapid decline of dihydroxylated catechins at 18 °C (Fig. 5). This corresponds to the subsequent decline in theaflavins after 120 min. Depletion of di- and trihydroxylated catechins followed a similar trend at 24 °C. Theaflavins declined after 90 min of fermentation at 24 and 30 °C. Correspondingly percent thearubigins increased.

Theaflavins formation or depletion was found to be dependent on both fermentation temperature and time. Raising fermentation temperature from 18 to 24 °C resulted in rapid formation of theaflavins for each clone. The peak concentration of the theaflavins was reached much faster at elevated temperatures for each clone. The decline in total theaflavins was rapid at 30 °C.

Table 4
Brightness (%) regressed against theaflavins and thearubigins.

BR %		Linear model			
Variable	DF	Parameter estimate	Standard error	*t* Value	Pr > |*t*|
Intercept	1	43.75	3.22	13.55	<.0001
TF	1	0.53	0.07	7.24	<.0001
TR %	1	−1.76	0.10	−16.42	<.0001
Root MSE		2.78			
Dependent mean		24.17			
Coeff var		11.51			
R-square		0.86			
Adj R-sq		0.86			
ANOVA					
Source	DF	Sum of squares	Mean square	*F*-Value	Pr > *F*
Model	2	5253.95	2626.97	338.74	<.0001
Error	105	814.28	7.75		
Corrected total	107	6068.23			

Table 5
Tasters' brightness regressed against theaflavins and thearubigins.

BR (tasters)		Linear model			
Variable	DF	Parameter estimate	Standard error	*t* Value	Pr > |*t*|
Intercept	1	7.39	1.30	5.67	<.0001
TF	1	0.14	0.03	4.74	<.0001
TR %	1	−0.25	0.04	−5.78	<.0001
Root MSE		1.12			
Dependent mean		6.10			
Coeff var		18.42			
R-square		0.55			
Adj R-sq		0.54			
ANOVA					
Source	DF	Sum of squares	Mean square	*F*-Value	Pr > *F*
Model	2	163.11	81.55	64.50	<.0001
Error	105	132.76	1.26		
Corrected total	107	295.87			

Table 6
Correlation Coefficients ($n = 108$).

	BR %	BR (tasters)
TF (μmol/g)	0.72	0.64
	<.01	<.01
TR %	−0.89	−0.67
	<.01	<.01

3.4. Relationship between total theaflavins, thearubigins and liquor brightness

Total theaflavins and thearubigin contents explained 86.58% of spectrophotometric brightness and 55.13% of tasters' scores for brightness. Theaflavins had a significant, positive effect on the brightness of the black tea liquors. However, thearubigins had a negative effect on brightness (Tables 4 and 5).

Theaflavins correlated positively with spectrophotometric brightness ($r = 0.7221$, $p < 0.01$), $r^2 = 0.5214$, compared to ($r = 0.6391$, $p < 0.01$) $r^2 = 0.4084$ for tasters' scores on brightness. This may be the reason for the determined spectrophotometric brightness of theaflavins 52.1% and the 40.8% brightness scored by the tasters. Thearubigins had a higher negative impact on Spectrophotometric brightness ($r = -0.8937$, $p < .01$), $r^2 = 0.7986$, than on tasters' brightness ($r = -0.6748$, $p < 0.01$), $r^2 = 0.4553$ (Table 6). This study showed a significant contribution of theaflavins to spectrophotometric and tasters' brightness (Table 5). Theaflavins content strongly correlated with spectrophotometric and tasters' brightness ($r = 0.7221$, and 0.6391 respectively, $p < 0.0001$). However, thearubigins had a negative and significant effect on spectrophotometric and tasters' brightness of black tea liquor ($r = -0.8938$ and -0.6748, respectively, $p < 0.0001$).

4. Discussion

During fermentation process theaflavins are continuously being formed or degraded (Robertson, 1983). As the substrate catechins are oxidized, then the degradation of teaflavins becomes more dominant. Raising the fermentation temperature increases enzymatic oxidation leading to a faster depletion of all catechins. A decline in total theaflavins content and liquor brightness with extended fermentation time and rise in temperature has been reported (Obanda et al., 2001). This particular study demonstrated an increase in percent thearubigins with increase fermentation temperature and duration. The rate of formation chemical quality parameters with fermentation temperature and time was clonal dependent.

Clonal variation in polyphenol oxidase activity has been observed to influence the rate of formation of the quality parameters (Obanda et al., 2001). Clone 6/8, a putative Assam clone has medium low EC content and 0.57 μmol/g dry matters, in fresh tea shoots (Magoma, Wachira, Obanda, Imbuga, & Agong, 2000). Clone 303/577 is an offspring of clone 6/8 with slightly higher EC content (0.61 μmol/g dry matter). Clone 303/577 was reported to have higher EGCg content in fresh tea shoots than clone 6/8. This probably explains why the two clones were not significantly ($p < 0.05$) different in the mean total theaflavins. The two clones also exhibited similar trends in theaflavins formation especially at 24 °C. The low levels of ECg (0.80 μmol/g) in clone 6/8 fresh leaf as reported by Magoma et al. (2000) could be another limiting factor to much higher theaflavins content. Though clone 303/577 had higher EGCg in fresh tea leaves than clone 6/8 (Magoma et al., 2000), the mean EGCg in the black tea was lower than clone 6/8 black tea. It was also reported in the same (Magoma et al., 2000) study that clone 303/577 had higher EC and ECg than clone 6/8. This implies that on fermentation the dihydroxylated catechins (EC and ECg) will be more limiting for theaflavins formation in clone 6/8 and therefore the higher residual EGCg content. The higher content of EGCg and EGC in clone 303/577 fresh tea shoots are depleted much faster due to a correspondingly higher content of EC and ECg. The trihydroxylated catechins were depleted faster in clone 303/577 than the other clones.

Clone 311/287 had the highest mean theaflavins content (26.99 ± 0.53 μmol/g). The clone was reported to have high EGCg content (1.95 μmol/g) and EGC (5.72 μmol/g) (Magoma et al., 2000). However the clone had a low EC (0.52 μmol/g) and ECg (1.02 μmol/g). The levels of EC and ECg in the fresh tea shoots was however not low enough to limit maximum formation of theaflavins (probably theaflavin-3′-gallate and theaflavin-3, 3′-gallate). Clone 311/287 fresh tea shoots were higher in EGC. This suggests that simple theaflavins and theaflavin-3′-gallate could also be present in the black tea in large proportions. This observation may account for the higher theaflavins content in clone 311/287 black tea compared to clones 303/577 and 6/8 at all temperature-time combinations. Initial high levels of EGC and EGCg in clone 311/287 shoots did not translate to a higher residual EGC and EGCg in black tea compared to clone 6/8. This may be due to the high potential of theaflavins formation in clone 311/287. It is however evident from the three clones studied that the level of dihydroxylated catechins is not limiting in theaflavins formation.

During black tea processing, the tea shoots are macerated to initiate fermentation, in which the enzyme polyphenol oxidase catalyses oxidation of catechins into quinones by molecular oxygen. The quinones from the oxidation of B-ring dihyroxylated catechins condense with quinones arising from the oxidation of B-ring trihydroxylated catechins to give different theaflavins (Robertson, 1992). The correct balance and amount of the trihydroxy-3-flavanols and dihydroxyflavan-3-ols is therefore necessary to ensure maximum formation of theaflavins (Wright et al., 2002). Due to differences in reduction potential, quinones also take part in redox equilibration reactions during fermentation, causing the different catechins to deplete at different rates (Bajaj, Anan, Tsushida, & Ikegaya, 1987). In this present study, after 60 min of fermentation trihydroxylated catechins were lower than dihydroxylated catechins for clone 6/8, indicating an initial fast depletion. The B-ring trihydroxylated catechins, (EGC and EGCg), are oxidised at a much faster rate than the B-ring dihydroxylated catechins: (EC, ECg) due to their lower redox potentials (Owuor & Obanda 2007). Though higher initially, they could be the limiting factor in theaflavins formation because they are depleted fast. However, the flavan-3-ols gallates have a higher substrate inhibition property on polyphenol oxidase than the non-gallated flavan-3-ols because of higher molecular weights and flexibility (Robertson, 1983).

The was an equal rate of depletion of di- and trihdroxylated catechins with a rise in theaflavins between 60 and 90 min of fermentation at 18 °C (Figs. 3 and 4). This is indicative of the importance of the ratio of the two groups of catechins in theaflavins formation which require equal concentrations. The low levels of either di- or trihydroxylated and the unequal rates of catechins depletion seems to lead to a decline in theaflavins content. The imbalance of simple quinones relative to gallocatechin quinones created by redox equilibration during coupled oxidation of catechins, is known to be key in directing the majority of the catechins particularly the gallocatechins into thearubigins fraction (Robertson, 1983). Theaflavin reduction could be increased by lower levels of catechins relative to gallocatechins in green tea shoots (Robertson, 1983). In the present study theaflavins content declined with increase in gallocatechins to catechins ratio.

Oxidative degradation of theaflavins has been shown to result in formation of thearubigins (Robertson 1992). This further explains the increase in percent thearubigins with fermentation time. High enzyme activity due to high substrate concentration at the early stages of fermentation causes inhibition of theaflavins formation due to a preferential demand for oxygen by the enzyme. Theaflavins is known to contribute to briskness and brightness of tea liquor whilst thearubigins are responsible for the colour and body of the liquor (Hilton & Ellis, 1972).

It was concluded that the experimental conditions tested in this study form a good basis for clonal specific processing conditions that can be utilised in manufacturing quality black tea.

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