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Changes in thearubigin fractions and theaflavin levels due to variations in processing conditions and their influence on black tea liquor brightness and total colour

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Abstract

Increase in coarser plucking standards significantly ($P<0.05$) depressed levels of theaflavin (TF; μmol/g), total colour and brightness (spectrophotometer) of black tea. But total thearubigins (TR) TRSI, TRSII and brightness (taster) levels did not significantly ($P<0.05$) change with plucking standards. Increasing fermentation duration led to rise in TF (μmol/g), total TR, total colour, TRSI and TRSII, liquor brightness (taster) but not liquor brightness determined by spectrophotometer method ($P<0.05$). Correlation analysis showed that total TR and TRSII had negative correlations ($r=-0.66$ and -0.77, $P<0.01$), respectively, while total TF levels had positive correlation with taster brightness ($r=0.57$, $P<0.01$). Total TF level had highest correlation with spectrophotometer liquor brightness ($r=0.87$, $P<0.01$) for a single substance. TRSII and total TR gave an r of 0.86 indicating that the two groups of substances were strongly correlated to each other. Regression analysis showed that the direct linear model gave the best fit for the sample data studied. The coefficient of multiple determination (R^2) was 0.606 in linear model I for the tasters' liquor brightness. Thus, the independent variables TF and total TR explained 60.6% of the total variation in liquor brightness scores observed. When TRSI and TRSII were included in the linear model II instead of total TR, but with TF maintained, the coefficient of multiple determination improved to 78.9%. This confirmed that the brightness attribute of black tea, assessed by the taster, could best be explained by the combination of TF and TRSII and that TRSI had a lesser role in the tasters' evaluation of liquor brightness. Indeed, the test statistic in linear model I showed that the coefficient of TF positively and significantly influenced liquor brightness at $P<0.01$ whereas the coefficient of total TR negatively and significantly influenced liquor brightness ($P<0.0001$). However, in linear model II the effect of total TR on the taster brightness was clearly unmasked and the influence of each individual component well elucidated. The coefficient of TRSII was negative and significantly explained liquor brightness at 0.01% level ($P<0.0001$). The coefficient of TRSI was negative but insignificant. Hence it has no discernible influence on the taster liquor brightness. The coefficient of TF was positive and significantly explained taster liquor brightness ($P<0.01$). Thus, TF and TRSII explain taster liquor brightness and the lower the level of TRSII the higher the score for brightness. The situation for spectrophotometer brightness was somewhat different. The coefficient of multiple determination was 0.896 in linear model I. Thus, TF and total TR explained 89.6% of the variation in spectrophotometer brightness. The coefficient of TF was positive whereas that of total TR was negative. Both coefficients were significant ($P<0.0001$). When TRSI and TRSII were included in the linear model II instead of total TR, R^2 improved to 91%. Unlike for the tasters' brightness, the coefficient of TRSI was now significant ($P<0.05$). The coefficient of TRSII was negative and significant ($P<0.01$) whereas the coefficient of TF was positive and significant ($P<0.0001$). The differences in the contributions of TRSI and TRSII to black tea liquor brightness and the observed discrepancy between the test methods, due to variations in plucking standards and fermentation duration, are discussed.

1. Introduction

During black tea processing fermentation, the endogenous flavanols in the fresh green tea leaf undergo various oxidative reactions catalyzed by enzymes, to produce stable components that include theaflavins (TF), thearubigins (TR) and other polymerization products (Roberts, 1962; Sanderson, Berkowitz, Co, & Graham, 1972). The mechanisms of formation and the chemical structures of TF are well elucidated (Bhatia, 1963; Brown, Falsaw, Haslam, Holmers, & Ollis, 1966;

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Takino, Imagawa, Harikawa, & Tanaka, 1964). TF contribute to the bright orange-red tone i.e. brightness and colour of black tea liquor (Sanderson et al., 1976). The formation and contribution of TR to quality characteristics of black tea have also been reported but their structures remain speculative. In 1961, Roberts and Smith demonstrated TR could separate into two large groups, i.e. TRSI and TRSII, due to differences in chemical polarities. Roberts (1962) reported that TR was acidic brown pigments formed by the oxidative degradation of TF. Brown, Eyton, Holmers, and Ollis (1969) suggested that TR were polymeric proanthocyanidins. Berkowitz, Coggon, and Sanderson (1971) reported that some TR are derived from theaflavic acids during fermentation. Robertson (1983) concluded that TR could form through many pathways during fermentation. Using a model system, Bajaj, Anan, Tsushida, and Ikegaya (1987) indicated that the predominant pathway for the formation of black tea chemical quality parameters depended on flavanol composition, oxygen concentration, the temperature and polyphenol oxidase activity during fermentation. Bailey, Nursten, and McDowell (1991) suggested that some of the TR could be polydisperse flavanol polymers incorporating chromophoric monomer units rather than proanthocyanidin polymers.

Apart from the ability to chemically separate easily, TRSI and TRSII also differ considerably in brown colour intensity and hence their contributions to total colour and possibly to liquor brightness of black tea. TRSI and TRSII are easily measured routinely without the need for sophisticated chromatographic-spectroscopic techniques (Bailey et al., 1991, 1992; Cattell & Nursten, 1977), that divide TR into many components of fairly similar spectral characteristics. Division of TR into many groups makes the elucidation of individual TR components' contribution to tea quality difficult (McDowell, Taylor, & Gay, 1995). Thus, the role of TR groups in black tea liquor brightness is not well understood. However, black tea liquor brightness and colour are influenced by various agronomic and processing conditions, such as plucking standards (Obanda & Owuor, 1995), handling of plucked leaf, withering and fermentation conditions of temperature (Owuor & Obanda, 1997), relative humidity and duration (Obanda, Owuor, & Mang'oka, 2001).

Black tea liquor brightness and colour are critical quality attributes used in tea trade to rank and price black teas (Biswas, Sarkar, & Biswas, 1973; McDowell, Feakes, & Gay, 1991). Usually, black tea liquor brightness and total colour, determined by the standard laboratory methods, correspond to tea tasters' rankings for the same attributes. However, we have repeatedly observed that liquor brightness, measured by the spectrophotometer (laboratory) method, does not always correspond to tea tasters' rankings, despite such black teas having similar levels of TF and total TR contents. These observations have led to the suspicion of significant differences between the spectrophotometer method and tasters' responses to the chemical groups present in black tea. This paper reports the changes observed in liquor brightness, measured by the spectrophotometer method and by the tea taster, due to variations in plucking standards, relative humidity, temperature and duration at the withering and fermentation stages of black tea processing. The probable differences in the relative contributions of TF and TR groups TRSI and TRSII to black tea liquor brightness and total colour, as perceived by measured by the tea taster and measured by the spectrophotometer method are discussed.

2. Materials and methods

Green tea shoots of two leaves and a bud were plucked from clone 6/8 planted in the Museum at the Timbilil estate of the Tea Research Foundation of Kenya, Kericho, situated at an altitude of 2178 m above the sea level, latitude 0° 22′S and longitude 35° 21′E.

2.1. Black tea manufacture

2.1.1. Effects of plucking standards and fermentation duration on black tea chemical and sensory parameters

The objective was to demonstrate differences in the influence of plucking standards and fermentation duration on the levels of liquor brightness and total colour of black tea. Clone 6/8 plot was demarcated to produce 1 leaf + bud, 2 leaves + bud, 3 leaves + bud, 4 leaves + bud and 5 leaves + bud samples for CTC processing after 18–21 h ambient withers. Fermentation was at 22 °C for 90 min before firing in a mini-fluid bed dryer. In another set, 2 leaves + bud samples, treated under similar wither conditions were fermented for 30, 60, 90, 120 and 150 min and fired. All treatments were replicated four times. All black tea samples were subjected to chemical analyses for TF, total TR, TR fractions, total colour and liquor brightness. The results are presented in Table 1.

2.1.2. Response of black tea chemical and sensory quality parameters to hot-dry conditions of wither

During black tea processing, the fresh green tea leaves are subjected to a period of withering necessary for the formation of quality. The temperature and relative humidity during withering have significant influence on black tea quality. Also, tea clones respond differently to hot and dry wither conditions. Thus, to cause variations in the levels of plain black tea quality parameters, two and a bud portions of the three tea varieties: *Camellia sinensis* var.*assamica* (clones 6/8, 31/8, 31/11), *Camellia sinensis* var. *assamica* ssp. *Lasiocalyx* (clones 301/4, 301/5, 301/6), and *Camellia sinensis* var. *sinensis* (clones 14/1,

Table 1
Variations of black tea chemical parameters due to plucking standards and fermentation time

	TF (μmol/g)	TR%	Total colour (%)	TRSI (%)	TRSII (%)	Spectrophotometer brightness	Tasters' brightness
(a) *Different plucking standards at 90 min fermentation duration*							
Plucking standard							
1+b	28.3	16.9	5.81	2.51	5.74	31.5	5.5
2+b	27.3	16.0	5.14	2.34	5.24	32.3	7.5
3+b	24.2	16.8	5.12	2.44	6.37	28.3	6.0
4+b	23.6	17.8	4.77	2.23	5.64	25.0	5.5
5+b	20.0	15.4	4.07	1.95	5.41	22.31	5.5
C.V.%	2.74	6.89	7.61	10.98	11.44	8.32	21.5
LSD $P \leqslant 0.05$	1.27	NS	0.71	NS	NS	4.37	NS
S.E.	0.301	0.51	0.17	0.13	0.32	1.04	0.58
(b) *Fermentation time at two leaves and a bud plucking standard*							
Fermentation time (min)							
30	10.7	11.9	2.44	0.65	2.34	29.6	9.0
60	21.4	14.6	4.29	1.61	4.03	31.1	8.5
90	27.3	16.1	5.14	2.33	5.38	32.3	7.5
120	25.9	18.2	5.76	2.52	6.66	29.5	5.5
150	23.1	15.9	5.09	2.84	6.96	28.9	5.0
C.V.%	20.5	10.2	14.9	8.36	11.2	10.15	17.3
LSD $P \leqslant 0.05$	8.36	2.95	1.27	0.26	0.87	NS	2.0
S.E.	1.991	0.701	0.302	0.08	0.28	1.380	0.551

St.14 and 83/1) were plucked, and withered under hot dry conditions, i.e. 36 °C dry bulb, 18 °C wet bulb, for 10, 20 and/or 35 h, respectively. The leaf was macerated using the crush, tear and curl (CTC) method using a mini-CTC machine. Fermentation was for 90 min at 22 °C. The fermented leaf 'dhool' was fired, using a miniature fluid bed drier to obtain black tea from which fibre was removed before being subjected to both chemical and sensory analyses without sorting. The results obtained are listed in Table 2.

The objective was to use different tea clones, temperature and humidity at withering to cause variation in the formation of black tea chemical and sensory quality parameters. The data were utilized in quantifying the relationship(s) between different thearubigin groups and liquor brightness and total colour, as perceived by the taster and/or measured by the spectrophotometer method.

2.2. Dry matter determination of black tea

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

2.3. Total TF content analysis (Flavognost)

Total TF were determined by the Flavognost method (Hilton, 1973). A tea infusion was made with 375 ml of boiling water, added from an overhead boiler into a tared flask, and 9 g of tea. The flask was shaken for 10 min, the infusion filtered rough cotton wool, and allowed to cool to room temperature, and then 10 ml were pipetted 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. Two millilitres of the upper layer were pipetted into a test tube, followed by 4 ml ethanol and 2 ml Flavognost reagent (2 g diphenylboric acid-2-aminoethyl ester dissolved in 100 ml ethanol). The contents were mixed and colour allowed 15 min to develop. The absorbance (A) at 625 nm was read against an IBMK/ethanol (1:1 v/v) blank.

$$\mathrm{TF}(\mu\mathrm{mol/g}) = \mathrm{A}_{625} \times 47.9 \times 100/\mathrm{DM}$$

2.4. Determination of liquor total colour

Five millilitres of filtered standard tea infusion from TF analysis were pipetted into 45 ml 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 a distilled water blank. The result was corrected for dry matter content of the black tea samples.

$$\text{Liquor colour} = (\mathrm{A}_{460\ \mathrm{nm}} \times 10)/(\mathrm{DM}/100)$$

2.5. Spectrophotometer measurements of total TR, TRSI and TRSII

The method of Roberts and Smith (1961) was used to determine total TR and TRSI and TRSII. Fifty

Table 2
Response of black tea chemical and sensory quality parameters due to different conditions of withering temperature and relative humidity for different tea varieties

Chemical parameter	*Camellia* variety	Clone	Wither (h) at 36 °C Dry bulb, 18 °C Wet bulb			Mean
			10	20	35	
Theaflavin (μmol/g)	*Camellia sinensis* var. *assamica*	6/8	25.3	24.2	12.6	20.7
		31/8	21.6	20.9	14.0	18.7
		31/11	21.5	20.5	17.6	20.0
	Camellia sinensis var. *assamica* ssp. *Lasiocalyx*	301/4	17.2	16.5	10.5	14.7
		301/5	29.1	21.6	10.6	20.4
		301/6	13.4	10.3	5.75	9.84
	Camellia sinensis var. *sinensis*	14/1	20.7	20.6	17.3	19.4
		St.14	20.2	17.7	13.6	17.2
		83/1	15.2	14.4	12.0	14.0
		Mean	20.5	18.5	12.7	
	C.V.%		17.24			
	LSD $P \leqslant 0.05$		Wither (A) 1.40 Clone (B) 2.42 (Wither × Clone)			
TR (%)	*Camellia sinensis* var. *assamica*	6/8	15.5	16.8	18.2	16.8
		31/8	14.2	14.3	16.0	14.8
		31/11	14.0	15.3	16.5	15.3
	Camellia sinensis var. *assamica* ssp. *lasiocalyx*	301/4	19.0	19.1	19.0	19.0
		301/5	17.5	18.8	17.8	18.0
		301/6	17.8	18.4	15.8	17.3
	Camellia sinensis var. *sinensis*	14/1	16.3	17.1	17.4	16.9
		St.14	16.2	15.8	16.0	16.0
		83/1	14.6	15.3	16.8	15.5
		Mean	16.1	16.8	17.1	
	C.V.%		8.56			
	LSD $P \leqslant 0.05$		Wither (A) 0.67 Clone (B) 1.16 (Wither × Clone) NS			
Total colour (%)	*Camellia sinensis* var. *assamica*	6/8	4.88	5.01	4.51	4.80
		31/8	4.26	4.22	4.34	4.27
		31/11	4.33	4.58	4.96	4.62
	Camellia sinensis var. *assamica* ssp. *lasiocalyx*	301/4	4.94	4.69	4.48	4.70
		301/5	5.80	5.68	4.84	5.44
		301/6	3.92	3.94	2.86	3.57
	Camellia sinensis var. *sinensis*	14/1	3.79	3.97	4.19	3.99
		St.14	4.15	4.09	3.67	3.97
		83/1	3.56	3.73	3.86	3.72
		Mean	4.40	4.43	4.19	
	C.V.%		12.54			
	LSD $P \leqslant 0.05$		Wither (A) NS Clone (B) 0.44 (Wither × Clone) NS			
Liquor brightness (%) (spectrophotometer)	*Camellia sinensis* var. *assamica*	6/8	35.8	32.3	21.0	29.7
		31/8	30.3	29.5	17.2	25.6
		31/11	30.9	25.3	20.5	25.6
	Camellia sinensis var. *assamica* ssp. *Lasiocalyx*	301/4	19.7	15.6	12.4	15.9
		301/5	30.6	23.0	14.8	22.8
		301/6	18.9	13.5	13.6	15.4
	Camellia sinensis var. *sinensis*	14/1	28.2	26.3	20.8	25.1
		St.14	28.3	25.7	23.6	26.0
		83/1	24.6	21.2	18.4	21.4
		Mean	27.5	23.6	18.0	
	C.V.%		19.45			

(*continued on next page*)

Table 2 (*continued*)

Chemical parameter	*Camellia* variety	Clone	Wither (h) at 36 °C Dry bulb, 18 °C Wet bulb			Mean
	LSD $P \leqslant 0.05$	Wither (A) 2.11 Clone (B) 3.66 (Wither × Clone) NS				
	Camellia sinensis var. *assamica*	6/8	6.3	6.3	3.0	5.2
		31/8	7.0	6.3	3.0	5.4
		31/11	7.0	7.0	3.7	5.9
Tasters brightness	*Camellia sinensis* var. *assamica* ssp. *lasiocalyx*	301/4	4.3	3.7	2.3	3.4
		301/5	3.7	1.7	1.7	2.3
		301/6	3.7	3.7	1.7	3.0
	Camellia sinensis var. *sinensis*	14/1	7.7	6.3	5.0	6.3
		St.14	5.7	5.7	5.7	5.7
		83/1	5.7	5.7	4.3	5.2
		Mean	5.7	5.1	3.4	
	C.V.%	35.08				
	LSD $P \leqslant 0.05$	Wither (A) 0.90 Clone (B) 1.56 (Wither × Clone) NS				
TRSI%	*Camellia sinensis* var. *assamica*	6/8	2.88	3.19	1.75	2.61
		31/8	2.62	3.15	2.23	2.67
		31/11	2.92	3.35	2.79	3.02
	Camellia sinensis var. *assamica* ssp. *Lasiocalyx*	301/4	3.41	3.74	2.68	3.28
		301/5	3.85	3.97	2.80	3.54
		301/6	3.22	2.93	1.86	2.67
	Camellia sinensis var. *sinensis*	14/1	2.55	2.37	2.25	2.39
		St.14	2.62	2.35	1.88	2.29
		83/1	2.23	2.19	2.14	2.18
		Mean	2.92	3.03	2.26	
	C.V.%	29.17				
	LSD $P \leqslant 0.05$	Wither (A) 0.38 Clone (B) 0.65 (Wither × Clone) NS				
TRSII (%)	*Camellia sinensis* var. *assamica*	6/8	6.42	7.20	8.54	7.39
		31/8	5.43	5.99	7.66	6.36
		31/11	6.03	6.35	8.12	6.83
	Camellia sinensis var. *assamica* ssp. *lasiocalyx*	301/4	8.58	9.11	8.78	8.82
		301/5	8.47	9.91	9.17	9.18
		301/6	7.76	7.74	5.89	7.13
	Camellia sinensis var. *sinensis*	14/1	5.55	6.52	7.41	6.49
		St.14	6.85	6.51	7.06	6.81
		83/1	6.18	5.90	7.41	6.50
		Mean	6.81	7.25	7.78	
	C.V.%	8.56				
	LSD $P < 0.05$	Wither (A) 0.52 Clone (B) 0.89 (Wither × Clone) 1.55				

Brightness Key: Very Bright—11: Bright—9: Fairly Bright—7: A little Bright—5: Dull—3: Very Dull—1.

millilitres of the cool, well-shaken and filtered standard tea infusion from TF analysis were mixed with 50 ml isobutylmethylketone (IBMK) and gently shaken to avoid formation of an emulsion. The layers were allowed to separate and 4-ml portion of the IBMK layer was taken and made to 25 ml with methanol in a volumetric flask (Solution A).

Two millilitre portions of the aqueous layer were diluted to 10 ml with distilled water and then to 25 ml with methanol (Solution B).

Twenty-five millilitres of the remaining initial IBMK layer were taken in a separate flask and mixed with 25 ml of 2.5% aqueous sodium hydrogen carbonate. The mixture was vigorously shaken before the layers were allowed to separate and the aqueous layer discarded. A 4-ml portion of the washed IBMK layer was made to 25 ml with methanol (Solution C).

Two millilitres of a saturated oxalic acid aqueous solution and 6 ml of water were 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 absorbancies A_A, A_B, A_C, A_D of solutions A, B, C and D at 380 and 460 nm were obtained using a CE 393 Cecil Digital grating spectrophotometer with distilled water as the blank.

Each black tea sample was extracted in triplicate for the determination of the TR fractions and the brightness levels.

2.6. Calculation of the levels of TR in black tea liquor

By following the earlier procedures for solvent partitioning of black tea liquor components and based on the fact that mean absorbance of the TR fractions at 380 nm was 0.733 (Roberts & Smith, 1961), the following equation for estimating total TR was derived:

At 380 nm:

$$\%\ \mathrm{TR(Total)} = (375 \times 0.02 \times 6.25[2A_D + A_A - A_C])/(0.733 \times 9 \times \mathrm{DM}/100)$$

But the value $A_A - A_C$ represents the absorbance due to the IBMK-soluble free acid TR of SI type for which $A^{0.2\%}{}_{460\ \mathrm{nm}} = 0.138$.

Thus, at 460 nm and following the above solvent-partitioning procedures:

$$\%\ \mathrm{TRSI} = (375 \times 0.02 \times 6.25[A_A - A_C])/(0.138 \times 9 \times \mathrm{DM}/100)$$

Similarly, the value A_B represents absorbance of the IBMK-insoluble TR of SII type and after acidification with oxalic acid changes to A_D. These acidified SII type TR have $A^{0.2\%}{}_{460\ \mathrm{nm}}$ of 0.233, and are more deeply coloured than the SI type (Roberts & Smith, 1961). Hence, at 460 nm:

$$\%\ \mathrm{TRSII} = (375 \times 0.02 \times 12.5\ A_D)/(0.233 \times 9 \times \mathrm{DM}/100)$$

Each black tea sample was extracted in triplicate.

2.7. Determination of spectrophotometer liquor brightness

At 460 nm:

$$\mathrm{Brightness}\ (\%) = (100 \times A_C)/(A_A + 2A_B)$$

2.8. Ranking of liquor brightness by taster

The randomly numbered black tea samples were subjected to liquor brightness ranking by an experienced tea taster. The following scales were used to rank the level of the parameter in the tea liquor: very bright—11, bright—9, fairly bright—7, a little bright—5, dull—3, very dull—1.

2.9. Statistical analysis

Statistical analysis was carried out on the chemical parameters of the black tea samples manufactured under varying wither duration and the tasters' scores. The analytical procedures used to establish relationships among chemical substances were correlation and regression analyses. A simple correlation indicates the strength of the linear relationship between two variables. The correlation procedure was administered on tasters' scores and spectrophotometer measurements of black tea quality parameters. Regression analysis was undertaken to determine the major substances that explain liquor brightness in both types of measurement. The dependent variable was liquor brightness or total colour. The independent explanatory variables were TRSI, TRSII, TR, and TF. The relationship between liquor brightness or total colour and the other variables was presented as:

$$\mathrm{LB} = f\ (\mathrm{TR},\ \mathrm{TF}) \quad (1)$$

$$\mathrm{LB} = f\ (\mathrm{TRSI},\ \mathrm{TRSII}, \mathrm{TF}) \quad (2)$$

$$\mathrm{TC} = f\ (\mathrm{TR},\ \mathrm{TF}) \quad (3)$$

$$\mathrm{TC} = f\ (\mathrm{TRSI},\ \mathrm{TRSII}, \mathrm{TF}) \quad (4)$$

where

LB, tasters' scores or spetrophotometric measurements of liquor brightness in a sample;
TRSI, levels of thearubigins I in a sample;
TRSII, levels of thearubigins II in a sample;
TR, levels of total thearubigins in a sample;
TF, levels of theaflavins in a sample; and
TC, total colour of a sample.

The first mathematical relationship was assumed to be linear. Afterwards, other relationships, such as log linear, partial linear and square root, were tried. The software used for analysis was SPSS/PC+. "Enter" selection procedure was adopted to compute the regression equations. This was necessary because the objective of the analysis was to determine the combination of substances that best explain liquor brightness. Therefore, all the explanatory variables had to be entered into the model.

3. Results and discussion

The effects of varying plucking standards and fermentation duration on black tea chemical and sensory parameters are presented in Table 1(a) and (b). Increase

in coarser plucking standards significantly ($P<0.05$) depressed levels of TF (μmol/g), total colour and spectrophotometer brightness of black tea [Table 1(a)]. But total TR, TRSI, TRSII and tasters' assessment of liquor brightness levels did not significantly ($P<0.05$) change with plucking standards [Table 1(b)]. Increasing fermentation duration led to rise in TF (μmol/g), total TR, total colour, TRSI and TRSII, liquor brightness as ranked by the taster [Table 1(b)]. But the same variation of fermentation time had non-significant ($P>0.05$) influence on liquor brightness as determined by spectrophotometer method. The results presented in Table 1(a) and (b) exemplified the changes usually observed, and the differences between test methods, for liquor brightness of black tea due to variations in the plucking standards and fermentation duration adopted at processing.

Clones and wither durations under hot and dry conditions (36 °C, dry bulb, 18 °C wet bulb) caused significant variations in most chemical and sensory quality parameters of black tea (Table 2). The longer the wither duration the lower the TF levels, but the greater the levels of total TR (%) in black teas, except for the ssp. *Lasiocalyx* tea variety, for which TR fell slightly at 35 h of wither (Table 2). The decline in TF levels within the 10–20 h wither interval was less than that recorded for the 20–35 h wither interval. Conversely, the rise in total TR (%) within the 10–20 h wither interval was less than that recorded within the 20–35 h wither interval. Changes were also recorded for TRSI and TRSII levels as withering progressed. The formation of TRSI declined significantly within the 20–35 h wither interval whilst TRSII formation showed the opposite response save for the *Camellia sinensis* var *assamica* ssp. *Lasiocalyx* clones (301/4, 301/5, 301/6) which had declining TRSII levels after 20 h of wither duration. The variations in levels of TRSI and TRSII in black tea due to wither conditions were clonal-dependent rather than tea variety dependent (Table 2).

Variation in wither duration did not produce significant changes ($P<0.05$) in total colour, but the changes due to clones were significant. For spectrophotometer liquor brightness, both variations due to clone and wither duration, produced significant ($P<0.05$) changes (Table 2). The longer the wither duration the less bright the black tea liquors became. For the same wither duration, the *Camellia sinensis* var *assamica* ssp. *Lasiocalyx*, clones (301/4, 301/5, 301/6), tended to have lower brightness levels in comparison with other varieties. Tasters' assessments of black tea liquor brightness levels produced similar results to those described for the spectrophotometer method (Table 2).

Correlation analysis was carried out with all the substances against the spectrophotometer and tasters' brightness of black teas, and the results are presented in Table 3. For tasters' brightness, TRSII produced the highest negative correlation ($r=-0.77$, $P<0.01$), followed by total TR ($r=-0.66$, $P<0.01$). Total TF levels had a positive correlation with tasters' brightness ($r=0.57$, $P<0.01$). For spectrophotometer brightness, total TR ($r=-0.57$, $P<0.01$) and TRSII ($r=-0.50$, $P<0.01$) had comparable correlations. Total TF levels had the highest significant correlation with spectrophotometer liquor brightness ($r=0.87$, $P<0.01$) for a single substance, representing an R^2 of 76% when compared with spectrophotometer liquor brightness, but only 32% for tasters' liquor brightness. It was thus evident that TF had a positive and major role in spectrophotometer brightness whilst TRSII had the same, but negative role in determining tasters' liquor brightness, possibly underlining the chemical basis of the differences between the test methods for liquor brightness. Tasters' brightness values correlated significantly with spectrophotometer brightness ($r=0.753$, $P<0.01$) and the R^2 of 57% suggesting that chemical factors responsible for tasters' brightness values could not account for about 43% of the variation in spectrophotometer brightness and vice versa.

TRSI did not appear to be major contributors to either test of liquor brightness as the coefficients r achieved were only −0.07 and 0.18, for tasters' brightness and spectrophotometer brightness, respectively.

TRSII and total TR each gave an r of 0.861, indicating that the two latter groups of substances were strongly correlated to each other (Table 3). Possible explanations are that TRSII has a larger proportion of

Table 3
Correlation coefficients (r) between TF, total TR, TRSI and TRSII, taster and spectrophotometer liquor brightness

Parameter	Tasters' brightness	Spectrophotometer brightness	TRSI	TRSII	Total TR	TF	Total colour
Tasters' brightness	1.000	0.753**	0.070	−0.765**	−0.658**	0.565**	−0.203
Spectrophotometeric brightness	0.753*	1.000	0.185	−0.496**	−0.569**	0.873**	0.254
TRSI	−0.070	0.185	1.000	0.436*	0.334	0.542**	0.738**
TRSII	−0.765**	−0.496**	0.436*	1.000	0.861**	−0.156	0.635**
Total TR	−0.658**	−0.569**	0.334	0.861**	1.000	−0.245	0.400*
TF	0.565**	0.873**	0.542**	−0.156	−0.245	1.000	0.598**
Total colour	−0.203	0.254	0.738**	0.635**	0.400*	6.598**	1.000

*Correlation is significant at the 0.05 level (two-tailed). **Correlation is significant at the 0.01 level (two-tailed).

total TR than does TRSI. The low r of 0.44 between TRSI and TRSII must mean that formation of the two groups of TR follow different pathways as fermentation progresses.

The results of regression analysis showed that the direct linear model gave the best fit for the sample data studied. These results were summarized in Tables 4 to 9. The coefficient of multiple determination (R^2) was 0.606 in linear model I for the tasters' liquor brightness (Table 4). This coefficient gives the proportion of the total variation in the dependent variable explained by the predictors included in the model. Thus, the independent variables TF and total TR explained 60.6% of the total variation in liquor brightness scores observed. When TRSI and TRSII were included in the linear model II (Table 5) instead of total TR, of course with TF maintained, the coefficient of multiple determination improved to 78.9%. This confirmed that the brightness attribute of black tea, assessed by the tasters' could best be explained by the combination of TF and TRSII and that TRSI has a lesser role in the tasters' evaluation of liquor brightness. Indeed, the test statistic in linear model I (Table 4) showed that the coefficient of TF positively and significantly influenced liquor brightness ($P<0.01$) whereas the coefficient of total TR negatively and significantly influenced liquor brightness ($P<0.0001$). However, in linear model II (Table 5), the effect of total TR on tasters' brightness values is clearly unmasked and the influence of each individual component well elucidated. The coefficient of TRSII was negative and significantly explained liquor brightness ($P<0.0001$). The coefficient of TRSI was negative but insignificant. Hence, it has no discernible influence on tasters' liquor brightness. The coefficient of TF was

Table 4
Tea liquor brightness (tasters') regressed against total TR and TF

Tasters' brightness	Linear model I				
Variable	β	S.E. β	Standard β	t	Sign.
Constant	12.959	2.864		4.525	0.0001
TF	0.145	0.045	0.430	3.250	0.003
TR	−0.644	0.154	−0.552	4.178	0.0001
Multiple R	0.778				
R^2	0.606				
Adjusted R^2	0.575				
Standard error	1.175				
ANOVA					
	Sum of squares	df	Mean square	F	Sign. F
Regression	50.907	2	25.454	18.447	0.0001
Residual	33.116	24	1.384		
Total	84.023	26			

TR, thearubigins; TF, theaflavin.

Table 5
Tea liquor brightness (tasters') regressed against TRSI, TRSII and TF

Tasters brightness	Linear model II				
Variable	β	S.E. β	Standard β	t	Sign.
Constant	9.373	1.268	–	7.394	0.0001
TF	0.162	0.045	0.479	3.594	0.002
TRSI	0.107	0.434	0.036	−0.245	0.808
TRSII	0.980	0.181	−0.675	5.421	0.0001
Multiple R	0.888				
R^2	0.789				
Adjusted R^2	0.762				
Standard error	0.877				
ANOVA					
	Sum of squares	df	Mean square	F	Sign. F
Regression	66.324	2	22.108	28.729	0.0001
Residual	17.699	23	0.770		
Total	84.023	26			

TR, thearubigins; TF, theaflavin.

positive and significantly explained tasters' liquor brightness ($P<0.01$). Thus, TF and TRSII explains tasters' liquor brightness and the lower the levels of TRSII, the higher the score for brightness.

The situation for spectrophotometer brightness was somewhat different. The coefficient of multiple determination was 0.896 in linear model I (Table 6). Thus, TF and total TR explained 89.6% of the variations in laboratory brightness. The coefficient of TF was positive whereas that of total TR was negative. Both coefficients were significant ($P<0.0001$). When TRSI and TRSII were included in the linear model II instead of total TR, R^2 improved to 91% (Table 7). Unlike for the tasters' brightness, the coefficient of TRSI was now significant ($P<0.05$). The coefficient of TRSII was negative and significant ($P<0.01$) whereas the coefficient of TF was positive and significant ($P<0.0001$). The differences in the contributions of TRSI and TRSII to black tea liquor brightness may help explain the observed discrepancy between the test methods due to variations in plucking standards and fermentation duration. Spectrophotometer brightness declined with increase in coarse plucking, largely because the levels of the positively contributing TF declined while the levels of the negatively contributing TRSI and TRSII remained fairly constant. For the same increase in coarse plucking, tasters' liquor brightness did not decline because the levels of TRSII remained fairly constant and the decline in TF levels were not large enough to influence tasters' evaluation of liquor brightness. On the other hand, spectrophotometer brightness levels did not significantly change as fermentation progressed [Table 1(b)], largely because the rises in TF levels was countered by rises in TRSI and TRSII. Conversely, tasters' brightness

Table 6
Tea liquor brightness (spectrophotometer) regressed against total TR and TF

Spectrophotometer brightness	Linear model I				
Variable	β	S.E. β	Standard β	t	Sign.
Constant	32.950	5.241		6.287	0.0001
TF	0.938	0.082	0.780	11.463	0.0001
TR	−1.567	0.282	−0.378	−5.551	0.0001
Multiple R	0.946				
R^2	0.896				
Adjusted R^2	0.887				
Standard error	2.150				
ANOVA					
	Sum of squares	df	Mean square	F	Sign. F
Regression	950.738	2		102.888	0.0001
Residual	110.886	24	475.369		
Total	1061.624	26	4.620		

TR, thearubigins; TF, theaflavin.

Table 7
Tea liquor brightness (spectrophotemetric) regressed against TRSI, TRSII and TF

Spectrophotometer brightness	Linear model II				
Variable	β	S.E. β	Standard β	t	Sign.
Constant	19.096	2.844		6.713	0.0001
TF	1.149	0.101	0.956	11.358	0.023
TRSI	−2.376	0.975	−0.225	−2.438	0.004
TRSII	−1.285	0.406	−0.249	−3.168	0.0001
Multiple R	0.957				
R^2	0.916				
Adjusted R^2	0.905				
Standard error	1.969				
ANOVA					
	Sum of squares	df	Mean square	F	Sign. F
Regression	972.495	3	324.165	83.651	0.0001
Residual	89.129	23	3.875		
Total	1061.624	26			

TR, thearubigins; TF, theaflavin.

Table 8
Total colour of black tea liquor regressed against total TR and TF

Total colour	Linear model I				
Variable	β	S.E. β	Standard β	t	Sign.
Constant	−1.216	0.926		−1.313	0.202
TF	0.089	0.014	0.740	6.167	0.0001
Total TR	0.242	0.050	0.582	4.846	0.0001
Multiple R	0.822				
R^2	0.675				
Adjusted R^2	0.648				
Standard error	0.380				
ANOVA					
	Sum of squares	df	Mean square	F	Sign. F
Regression	7.195	2	3.598	24.931	0.0001
Residual	3.463	24	0.144		
Total	10.659	26			

TR, thearubigins; TF, theaflavin.

Table 9
Total colour of black tea liquor regressed against TRSI, TRSII and TF

Total Colour	Linear model II				
Variable	β	S.E. β	Standard β	t	Sign.
Constant	0.071	0.306		0.232	0.819
TF	0.082	0.011	0.677	7.496	0.0001
TRSI	0.063	0.105	0.059	0.596	0.557
TRSII	0.370	0.044	0.716	8.481	0.0001
Multiple R	0.950				
R^2	0.903				
Adjusted R^2	0.891				
Standard error	0.212				
ANOVA					
	Sum of squares	df	Mean square	F	Sign. F
Regression	9.627	3	3.209	71.518	0.0001
Residual	1.032	23	0.045		
Total	10.659	26			

TR, thearubigins; TF, theaflavin.

values declined as fermentation proceeded because the levels of TRSII, which dictates far more strongly and negatively (i.e. TRSII coefficient = −0.980 compared to +0.162 for TF), rose with increase in TF levels.

Correlation analysis showed that TF, total TR, TRSI and TRSII all contributed positively towards total colour of black tea liquor. TRSI correlated most with total colour with r of 0.74 ($P<0.01$), representing an R^2 of 54% when compared to the correlations of TF and TRSII that were lower (Table 3). The results of regression analysis for total colour had a multiple determination coefficient of 0.675 in linear model I (Table 8). Therefore, TF and total TR explained 67.5% of the variations in total colour. The coefficients of both variables positively and significantly influenced total colour ($P<0.0001$). The inclusion of separate terms for TRSI and TRSII as independent variables in linear model II (Table 9) improved the proportion of variation in total colour explained to 90%. The coefficients of TF and TRSII were positive and significant at $P<0.0001$ whereas the coefficient of TRSI was positive but had no discernible influence on total colour. Thus, the substances that determine total colour are TRSII and TF.

4. Conclusions

Although the two methods for measuring liquor brightness respond in similar ways to the black tea chemical parameters of TF, TRSI and TRSII, differences arise from the relative magnitude in the contributions due to each parameter. For the spectrophotometer method, the differences in contributions between the three parameters are not as large as that for the taster. TRSII (coefficient = −0.980) has a proportionately larger influence than TF (coefficient = +0.162) on black tea liquor brightness when evaluated by the taster.

A better prediction of liquor brightness and total colour of black tea will be achieved with inclusion of TF, TRSI and TRSII than could be obtained with TF and total TR alone.

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