Changes in the chemical and sensory quality parameters of black tea due to variations of fermentation time and temperature

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Abstract

The changes in theaflavins and residual catechin compositions, thearubigins TRSI and TRSII, sensory characteristics of total colour, brightness and briskness of black tea were investigated. It was demonstrated that the degradation of individual theaflavins varied during fermentation. Decline in the levels of individual theaflavins was influenced by both duration and temperature and coincided with decline in brightness and briskness. ECG and EGCG were the main residual catechins in black tea. The formation of thearubigins TRSI and TRSII, differed in their response to fermentation duration and temperature. The effects of processing parameters, fermentation temperature and duration, on chemical quality and tea liquor sensory characteristics are discussed. © 2001 Elsevier Science Ltd. All rights reserved.

1. Introduction

Sensory quality characteristics of black tea are assessed, using either sight, smell, and/or taste of the beverage (Cloughley, 1981; Ding, Kuhr, & Engelhardt, 1992; Sanderson et al., 1976). Several studies have demonstrated linkages between the sensory quality characteristics of resultant black tea, the chemical composition of the green leaf and the black tea processing parameters (Bendall 1959; Biswas, Biswas, & Sarkar, 1973; Roberts & Smith, 1963). At present, it is agreed that catechins, the major polyphenols in the green tea shoots, together with their oxidation products, are responsible for most of the sensory characteristics associated with black tea liquors (Biswas et al., 1973; Roberts & Smith, 1963; Sanderson et al., 1976). The major tea leaf catechins include (−)epicatechin (EC), (−)epicatechin gallate (ECG), (+)catechin (+C); (−)epigallocatechin (EGC) and (−)epigallocatechin gallate (EGCG; see Fig. 1). The catechins differ in chemical structures, reduction potentials (Bajaj, Anan, Tsushida, & Ikegaya, 1987) and contribution to the astringent taste of tea (Ding et al., 1992; Kuhr & Engelhardt, 1991) and can be categorized, either according to the number

of hydroxyl groups on the B-ring ((+) C, EC and ECG are B-ring dihydroxylated while EGC and EGCG are B-ring trihydroxylated) or as gallated or non-gallated catechins [(−) EC, (−) EGC and (+) C are simple, non-gallated tea catechins while ECG and EGCG are gallated catechins; Sanderson, 1972; Sanderson & Graham, 1973]. Apart from their role in tea quality, catechins are increasingly being identified with a number of diverse properties of benefit to human health (Apostolides & Weisberger, 1995; Cheng et al., 1986, 1991; Obanda, Owuor, & Taylor, 1996; Sano et al., 1991). Flavonol glycosides are also present in the green tea leaf and are believed to contribute substantially to tea liquor colour (McDowell, Feakes, & Gay, 1990).

During black tea processing, the tea shoots are macerated to initiate fermentation, in which the enzyme polyphenol oxidase catalyzes oxidation of catechins into quinones by molecular oxygen (Bendall, 1959). The quinones from the oxidation of B-ring dihydroxylated catechins condense with quinones arising from the B-ring trihydroxylated catechins to give different theaflavins (Brown et al., 1966, 1969; Takino et al., 1964). Due to differences in reduction potential, quinones will also take part in redox equilibration reactions during fermentation, causing the different catechins to deplete at different rates (Bajaj et al., 1987). The B-ring trihydroxylated catechins, (EGC and EGCG), deplete at

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much faster rates than the B-ring dihydroxylated cate-chins: [EC, ECG, (+) C]. Catechins are also different in their degree of astringency (Ding et al., 1992; Sanderson et al., 1976). The simple, non-gallated tea catechins (−) EC, (−) EGC and (+) C are not as astringent as the gallated catechins ECG and EGCG. Thus, the catechin composition changes as fermentation proceeds; the taste and colour characteristics of black tea also change (Owuor & Obanda, 1993, 1998).

The oxidation of catechins occurs through enzyme-catalyzed reactions to form theaflavins and thear-ubigins. Theaflavins are bright and orange-red while thearubigins are more chemically heterogeneous and tend to be brownish-red. (Brown, Falshaw, Haslam, Hohnes, & Ollis, 1966; Brown, Eyton, Hohnes, & Ollis, 1969; Deb & Ullah, 1968; Takino, Imagawa, Harikawa, & Tanaka, 1964). Together, these compounds and fla-vonol glycosides (McDowell, Feakes, & Gay, 1991) give black tea liquor most of its taste and colour. Four major individual theaflavins are commonly formed during black tea processing (see Fig. 1). These include simple theaflavin (TF), theaflavin -3-monogallate (TF-3-MG), theaflavin −3′-monogallate (TF-3′-MG) and theaflavin-3, 3′-digallate (TFDG).

Theaflavins have astringent tastes, and contribute to the briskness of black tea (Deb & Ullah, 1968). Thea-flavin digallate is the most astringent and has been esti-mated to be 6.4 times more astringent than simple

theaflavin, and 2.88 times more astringent than either theaflavin-3-monogallate or theaflavin -3′-monogallate (Sanderson et al., 1976). Owuor and Obanda (1995) reported a normalized factor, namely theaflavin digal-late equivalents relating theaflavin composition to the astringency of black tea.

Studies on the effects of processing parameters on black tea chemical and sensory quality have demon-strated a decline in the levels of total theaflavins, liquor brightness and briskness, with extended fermentation time and rise in temperature (Deb & Ullah, 1968; Owuor & McDowell, 1994; Sanderson et al., 1976). However, the total thearubigins tend to respond to these parameters in the opposite manner to that observed for theaflavins. The extent of response of sensory and che-mical quality parameters to fermentation temperature and time has been clonal-dependent, implicating the influence of green leaf catechin composition and/or dif-ferences in polyphenol oxidase activity (Obanda & Owuor, 1992; Thanaraj & Seshadri, 1990). Irrespective of processing conditions, tea clones with high total phenolic content do not always produce black teas with the most theaflavins and thearubigins or black tea liquors with most colour, briskness and brightness (Hil-ton & Palmer-Jones, 1973; McDowell et al., 1991; Obanda, Owuor, & Taylor, 1997). Further, not all theaflavins or groups of thearubigins are formed at the same rate and to same extent under the same black tea
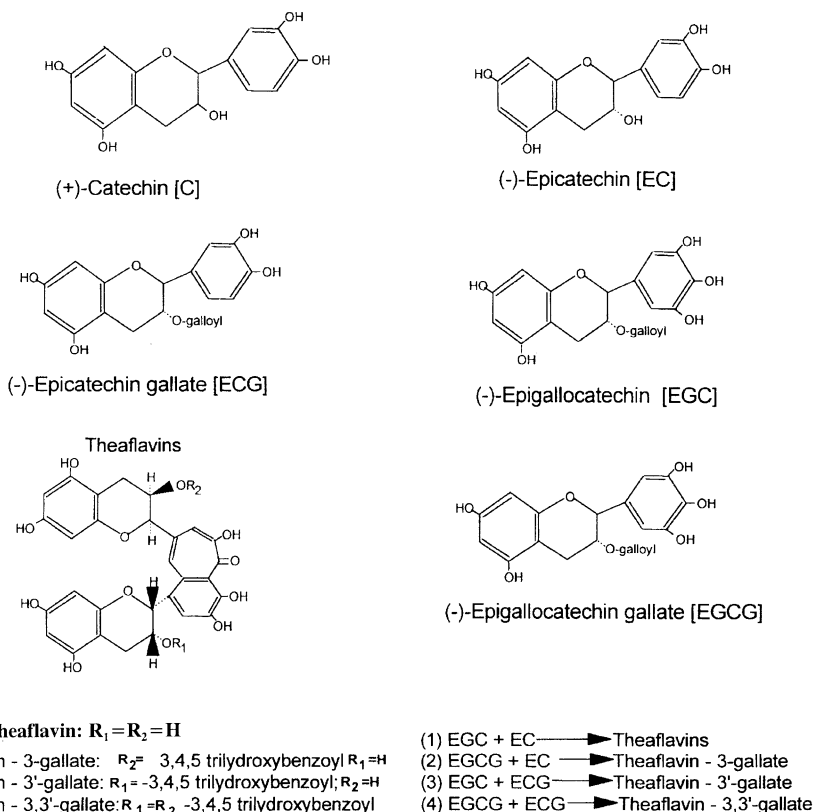

Fig. 1. Structures and general scheme for the formation of individual theaflavins.

processing conditions (Owuor & McDowell, 1994; Owuor & Reeves, 1986). The objective of this study was to use a single clone to investigate the response of residual catechin levels, the individual theaflavins, the two broad thearubigin groups TRSI and TRSII, as well as sensory changes in liquor briskness, brightness and colour to variations in fermentation time and temperature. The goal of this study was to explain the influence of green leaf catechin composition on sensory quality characteristics of resultant black tea.

2. Materials and methods

2.1. Tea manufacture

Leaf for miniature manufacture was obtained from a field of clone 6/8 at the Timbilil Estate of the Tea Research Foundation of Kenya at an altitude of 2178 m amsl and latitude $0°22''$ S. Thirty-two kilograms of young shoots, comprising about 70% two leaves and a bud, plus minor amounts of three leaves and a bud and loose leaf were plucked. The plucked leaf was allowed to wither under ambient conditions for 16–20 h and then miniature 'Crush, Tear and Curl' (CTC)-macerated (Owuor & Reeves, 1986). The macerated leaf was fermented for 60, 90, 120 and 150 min at either 20 °C (Wet bulb and Dry bulb) or 30 °C (Wet bulb and Dry bulb), using an environment control cabinet (TeaCraft, UK). The fermentation was terminated by drying the dhool to a moisture content of about 3% using a miniature fluid bed drier (TeaCraft, UK) set at 120 °C inlet and 80 °C exhaust air temperature. Each treatment was carried out on five occasions, thus giving five replicates.

2.2. Dry matter determination of black tea

Fifteen grams of black tea, weighed to the nearest 0.001g, 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 theaflavins content analysis (Flavognost)

From each clone was plucked 1200 g two and a bud green leaf which was then withered to achieve physical wither in 21 h. The leaf was macerated using the CTC method and fermented at ambient temperature of 22–24 °C for 90 min. The fermented leaf 'dhool' was fired using a miniature fluid bed drier and subjected to theaflavin analyses without sorting. Total theaflavin were determined by the Flavognost method (Hilton, 1973) as follows:

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 through 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 of ethanol and 2 ml of Flavognost reagent (2 g diphenylboric acid-2-aminoethyl ester dissolved in 100 ml of ethanol). The contents were mixed and colour was allowed 15 min to develop. The absorbance (A) at 625 nm was read against an IBMK/ethanol (1:1 v/v) blank.

$$\text{Theaflavin (}\mu\text{mol/g)} = A_{625}\times47.9\times100/\text{DM}$$

2.4. Determination of liquor total colour

Five millilitres of filtered standard tea infusion from theaflavin 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 a distilled water blank. The result was corrected for dry matter content of the black tea samples.

$$\text{Liquor colour} = (A_{460\text{nm}}\times10)/(\text{DM}/100)$$

2.5. Spectrophotometric measurements of total thearubigins and fractions TRSI, TRSII

The method of Roberts and Smith (1963) was used to determine total thearubigins and thearubigin TRSI and TRSII. Fifty millilitres of the cool, well-shaken and filtered standard tea infusion from theaflavin analysis were mixed with 50 ml of isobutylmethyl ketone (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 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 absorbances 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 thearubigin fractions and the brightness levels.

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

By following the above procedures for solvent partitioning of black tea liquor components and, based on the fact that mean absorbance of the thearubigin fractions at 380 nm was 0.733 (Roberts & Smith, 1961, 1963), the following equation for estimating total thearubigins was derived:

At 380 nm:

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

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

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

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

Similarly, the value A_B represents absorbance of the IBMK-insoluble thearubigins of SII type and, after acidification with oxalic acid, this changes to A_D. These acidified SII type thearubigins have $A^{0.2\%}_{460\,nm}$ of 0.233, and are more deeply coloured than the SI type (Roberts & Smith, 1961, 1963).

Hence, at 460 nm:

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

Each black tea sample was extracted in triplicate.

2.7. Determination of liquor brightness (spectrophotometric)

At 460 nm:
Brightness (%) = (100 x A_C) / (A_A + 2A_B)

2.8. Determination of the ratios of the individual theaflavins

The ratios of individual theaflavins were determined by HPLC (McDowell et al., 1991; Steinhaus & Engelhardt, 1989). For HPLC analysis, liquors were prepared by adding 4 g of black tea to 195 ml deionised water that had just reached the boil, and shaking for 10 min in a 475 ml capacity thermos flask. Clean liquors were obtained by filtration through cotton wool into a conical flask. The hot liquors were cooled to room temperature by placing the flasks containing the liquors under a cold water tap (1–3 min). The liquor was diluted (1:1) with double distilled water prior to HPLC analyses. The analyses were done using a Cecil Series 1000 HPLC with a 20 μl sample loop and a Hypersil 5μ ODS Column (25 cm×4.6 mm). The UV monitor was set at 375 nm and results recorded and analysed using a JCL6000 Cecil data system. Solvent A was 1% aqueous acetic acid and solvent B was acetonitrile. A linear gradient from 8 to 31% solvent B over 60 min with a flow rate of 1.5 ml/min was used (Bailey, McDowell, & Nursten, 1990). The total amount of theaflavins was allocated to the individual theaflavins, according to the ratios determined by HPLC, assuming the molar absorption coefficients of the four theaflavins are similar at 375 nm (Steinhaus & Engelhardt, 1989).

2.9. Calculation of astringency in theaflavin digallate equivalents

Owuor and McDowell (1994), using the Sanderson et al. (1976) astringency factors for the individual theaflavins, reported an improved TFDG equivalent, normalizing the contributions of individual theaflavins towards black tea astringency as follows:

TFDG equivalent of total TF(μmoles/g)

$$= TF/6.4 + TFMG \times 2.22/6.4 + TFDG$$

2.10. HPLC analysis of catechins in black tea

Standard tea infusion was filtered through a filter cartridge (DIS MIC 13HP, Advantec Toyo, Tokyo, Japan) before analysis on a Shiseido Capcell, C18 UG 120, 5-μm 4.6×250 mm HPLC column maintained at 35 °C. The mobile phase (A) was made up of 0.1% phosphoric acid in water and mobile phase (B) was 100% acetonitrile. The HPLC running programme was 0–5 min, 10% B; 5–25 min, 25% B; 25–26 min, 100% B; 26–35 min, 100% B; 35–45 min, 10% B. Flow rate was 1 ml/min, 10 μl injection volume and monitored at 270 nm, 0.05 AUFS. Authentic flavanol standards of (+) catechin C; (−) epicatechin EC; (−) epigallocatechin EGC; (−) epicatechin gallate ECG; (−) epigallocatechin gallate EGCG were used to identify peaks and calculate the concentrations of flavanols in black tea samples. Identified HPLC peaks with areas below 200 units were not quantified, and were recorded as not detectable. The HPLC peak for EC was not resolved from that due to EGCG in black tea. Separate spiking trials showed that

ECGC had a much larger absorbance/concentration ratio than EC, suggesting that EGCG contributed disproportionately more to the unresolved peak for both catechins. Hence, the variations due to EC in black teas were not followed.

2.11. Ranking of liquor brightness and briskness by taster

Randomly-numbered black tea samples were subjected to testing by an experienced tea taster for liquor

Table 1
Effect of fermentation duration and temperature on black tea chemical and sensory parameters

Parameter	Fermentation time (min)	Fermentation temperature (°C)		Mean
		20	30	
	60	22.6	24.1	23.4
Total TF (µmoles/g)	90	28.1	21.5	24.8
	120	27.2	19.7	23.5
	150	24.7	15.3	20.0
	Mean	25.7	20.2	
	C.V.%	5.58		
	LSD $P<0.05$	Fermentation time 1.33		Temp. 0.94
		Time×Temp. 1.88		
	60	16.6	19.3	17.9
	90	18.7	20.6	19.6
Total TR%	120	19.9	22.4	21.1
	150	19.8	20.7	20.3
	Mean	18.7	20.7	
	C.V.%	4.63		
	LSD $P<0.05$	Fermentation time 0.95		Temp. 0.67
		Time×Temp. 1.34 (NS)		
	60	2.49	3.76	3.12
	90	3.59	3.99	3.79
TRSI%	120	4.03	4.11	4.07
	150	4.03	3.54	3.78
	Mean	3.53	3.85	
	C.V.%	9.88		
	LSD $P<0.05$	Fermentation time 0.38		Temp. 0.27
		Time×Temp. 0.54		
	60	5.13	7.80	6.47
	90	6.80	9.79	8.29
TRSII%	120	7.79	11.1	9.44
	150	9.02	11.4	10.2
	Mean	7.18	10.0	
	C.V.%	10.2		
	LSD $P<0.05$	Fermentation time 0.91		Temp. 0.64
		Time×Temp. (NS)		
	60	30.5	26.2	28.3
	90	31.8	22.5	27.1
BR%	120	28.4	19.2	23.8
	150	26.2	17.4	21.8
	Mean	29.2	21.3	
	C.V.%	9.22		
	LSD $P<0.05$	Fermentation time 2.42		Temp. 1.71
		Time×Temp. NS		
	60	9.4	5.8	7.6
	90	7.8	4.6	6.2
Brightness (taster)	120	6.6	3.4	5.0
	150	6.2	1.8	4.0
	Mean	7.5	3.9	
	C.V.%	26.65		
	LSD $P<0.05$	Fermentation time 0.67		Temp. 0.47
		Time×Temp. (NS)		

Table 1 (*continued*)

Parameter	Fermentation time (min)	Fermentation temperature (°C)		Mean
		20	30	
	60	4.62	5.47	5.04
	90	5.47	5.88	5.67
Total colour	120	6.04	6.24	6.14
	150	5.92	5.79	5.86
	Mean	5.51	5.85	
	C.V.%	4.84		
	LSD $P < 0.05$	Fermentation time 0.29		Temp. 0.20
		Time×Temp. 0.40		
	60	8.3	5.3	6.8
	90	7.3	5.3	6.3
Briskness (taster)	120	6.7	5.0	5.8
	150	5.7	3.7	4.7
	Mean	7.0	4.8	
	C.V.%	34.6		
	LSD $P < 0.05$	Fermentation time NS		Temp. 1.2
		Time×Temp. (NS)		

brightness and briskness ranking. The following scales were used to rank the levels of these parameters in the tea liquor: very bright–11, bright–9, fairly bright–7, a little bright–5, dull–3, very dull–1; very brisk–11, brisk–9, fairly brisk–7, a little brisk–5, soft–3, very soft–1.

3. Results

The variation in black tea chemical and sensory quality parameters due to fermentation duration and temperature are given in Tables 1 and 2 and Fig. 2. At 20 °C fermentation temperatures, changes in total theaflavin content, with increase in fermentation time, between 60 and 150 min, showed a quadratic trend, while at 30 °C and within the same fermentation time range, total theaflavin content started at a slightly higher level but declined with increase in fermentation duration. Except at the 60 min fermentation interval, the 20 °C fermentation temperature produced significantly higher amounts of total theaflavins ($P \leqslant 0.05$) than the 30 °C fermentation temperature. Hence, there were significant interactions ($P \leqslant 0.05$) in the effects of duration and temperature on the formation of theaflavins. Fig. 2 shows the variations in the levels of individual theaflavins and theaflavin digallate equivalents due to fermentation temperature and duration. The impact of fermentation temperature on individual theaflavin formation increased with rise in fermentation time, such that, after 150 min, the 30 °C fermentation gave significantly lower levels ($P \leqslant 0.05$) of each individual theaflavin than the 20 °C fermentation. Similar observations were made on the impact of fermentation temperature and time on theaflavin digallate equivalents of black tea.

Total thearubigin (%) content responded differently to the fermentation conditions of temperature and duration. At the 20 °C fermentation temperature, formation of thearubigins was significantly slow and peaked at significantly lower levels at 20 °C than the 30 °C fermentation temperature.

The thearubigin groups, TRSI and TRSII, responded differently to changes in fermentation duration and temperature. The longer the fermentation time, the more TRSII were formed and showed little signs of peaking even after 150 min of fermentation, irrespective of fermentation temperature. TRSI formation reached a maximum, forming a plateau after 120 min at 20 °C fermentation temperature. At 30 °C, TRSI formation followed a quadratic trend with rise in fermentation duration, to peak between 90 and 120 min of fermentation.

Liquor brightness (spectrophotometric) responded to both fermentation temperature and duration. At 20 °C fermentation temperature, liquor brightness levels were significantly higher than at 30 °C fermentation for the same duration. Also, liquor brightness (%) showed a declining trend with increase in fermentation duration, but the decline was greater for the warmer temperature than the cooler temperature. Changes in liquor brightness (%) were such that black tea fermented for 60 min at 30 °C had the same brightness level as that for 150 min fermentation at 20 °C. Liquor brightness, assessed by tea tasters, showed a similar response due to fermentation temperature and duration as described for the laboratory method.

Changes in total colour of black tea liquors, due to both fermentation temperature and fermentation duration, followed a similar pattern to that observed for total thearubigin (%) content. The 20 °C fermentation
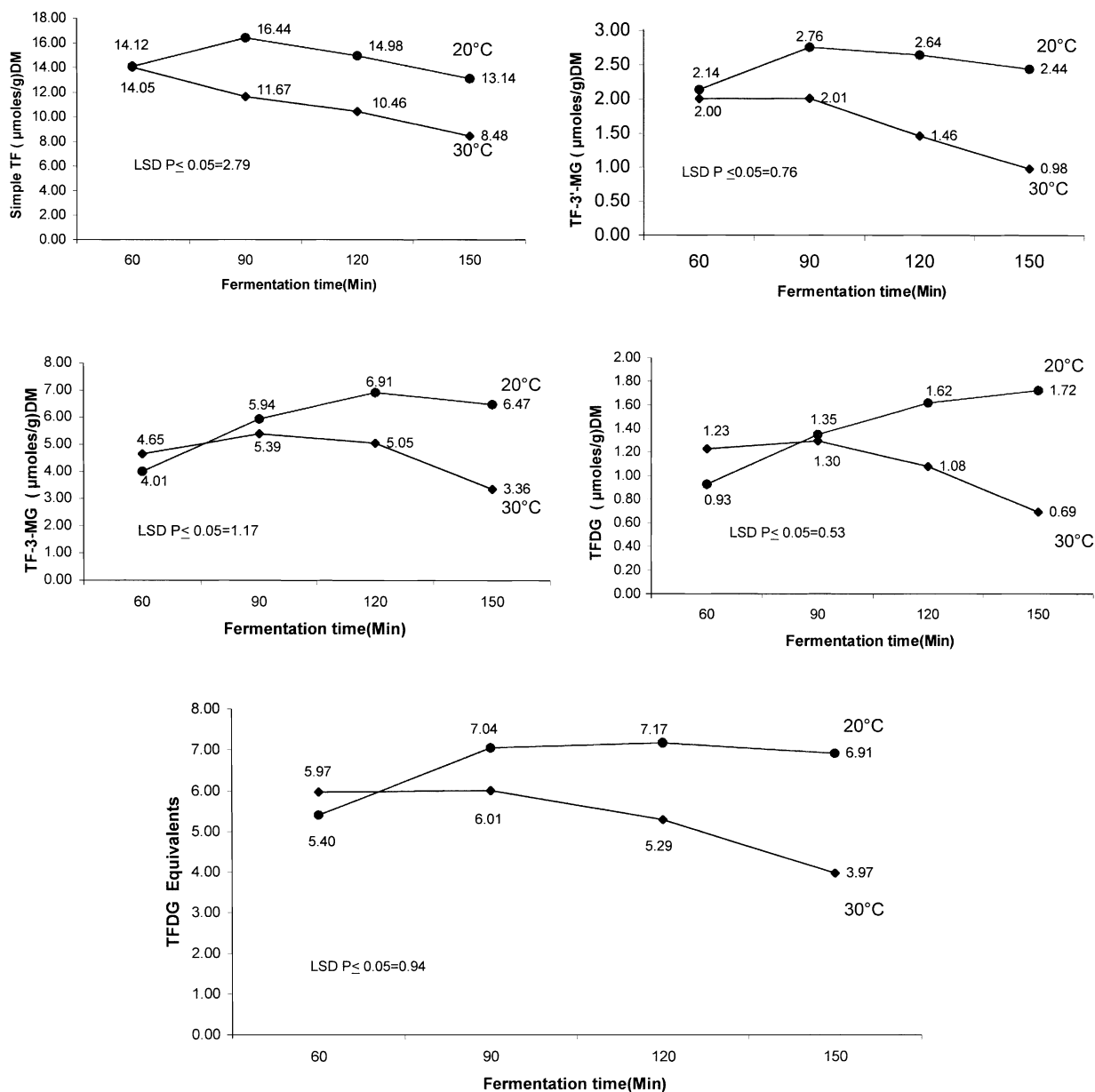
Fig. 2. Effect of fermentation duration and temperature on the individual theaflavin content and theaflavin digallate equivalents of black tea.

produced black tea liquors with lower colour levels than the 30 °C fermentation temperature for the same fermentation duration.

The briskness of tea liquor declined with fermentation time, irrespective of temperature. However, black teas fermented at 20 °C were more brisk and/or astringent than those made at 30 °C for the same fermentation duration. The differences in liquor briskness due to fermentation temperature were more significant than those due to fermentation duration.

Table 2 shows the changes in residual catechin levels at different fermentation durations and temperatures. All catechin levels declined with increase in fermentation time and temperature. At the 20 °C fermentation temperature, ECG, EGCG and gallic acid were present

in black tea at considerable levels even after 120 min of fermentation. However, residual levels of EGCG declined faster than ECG with rise in fermentation temperature and time.

4. Discussion

Raising fermentation temperature from 20 to 30 °C resulted in rapid formation of theaflavins after 60 min of fermentation. However, as fermentation proceeded, the levels of total theaflavins declined much more for the warmer temperature than the cooler temperature. It is believed that, during the fermentation process, theaflavins are continuously being formed and/or degraded

(Robertson, 1983). As the substrate catechins are depleted, then the degradation of theaflavin becomes dominant. As would be expected, raising the temperature speeded up the fermentation reactions, leading to faster depletion of all catechins. Although the individual theaflavin levels declined with extended fermentation duration and increased temperature, the results in this investigation demonstrated that the individual theaflavin levels declined to varying degrees. For instance, at 20 °C fermentation temperature, simple theaflavin and theaflavin-3′-monogallate levels showed comparatively minimal change, but theaflavin-3-monogallate increased from approximately 4.0 to 6.5 μmoles/g, and theaflavin 3,3′-digallate increased from 1.0 to 1.7 μmoles/g, respectively, over the 60–150 min fermentation duration. Further, the rate of change in individual theaflavin levels, with fermentation time at 20 °C, was different from that at the 30 °C fermentation temperature. At the latter temperature, simple theaflavin levels declined from 14.1 to 8.5 μmoles/g between 60 and 150 min of fermentation. But the other theaflavins showed much less decline, ranging between 0.5 and 1.5 μmoles/g over the same time interval at this temperature. These results demonstrate that the direction of change in theaflavin levels is both temperature- and time-dependent. At 90 min fermentation interval there were significant differences in simple theaflavin levels, due to temperature. It is unlikely that, after 60 min of fermentation, simple theaflavins were being formed through catechin oxidation, because one of the two catechins involved in its formation, EGC, was demonstrated to be almost exhausted (Table 2). However, it was still probable that simple theaflavin formation continued through possible hydrolytic breakdown of gallates. However, if such reactions were taking place, their contribution to formation of simple theaflavins were masked by the coupled oxidative reactions in which theaflavins polymerize, probably, to thearubigins (Bajaj et al., 1987; Opie, Clifford, & Robertson, 1993). It is also possible that formation of TF-3′-MG, through catechin oxidation, was complete after 60 min of fermentation since EGC, its precursor, was exhausted. Any further formation of TF-3′-MG was likely to be as a result of hydrolytic breakdown of TFDG. Hence, increasing fermentation temperature and duration beyond 60 min must have led to more degradation of the simple TF and TF-3′-MG rather than their formation. It was demonstrated that, at 20 °C fermentation, TFDG and TF-3-MG levels continued to rise after 60 min of fermentation, probably because their precursors, ECG and EGCG and possibly EC, were still present (Fig. 2, Table 2). However, raising fermentation temperature to 30 °C led to rapid decline in EGCG, ECG and the two theaflavins, TF-3-MG and TFDG.

At the 20 °C fermentation temperature, both TRSI and TRSII increased with rise in fermentation time but showed a different response at 30 °C. Unlike TRSI,

Table 2
Residual levels of flavanols (μg/g DM) in black tea produced at different fermentation intervals and temperatures[a]

Chemical parameter		Gallic acid		(−) EGC		(+) Catechin		Caffeine		(−)EGCG		(−)ECG	
Fermentation temperature		20 °C	30 °C	20 °C	30 °C	20 °C	30 °C	20 °C	30 °C	20 °C	30 °C	20 °C	30 °C
Fermentation time	60 min	2295(±80)	1798(±1337)	nd	nd	2315(±1548)	nd	16637(±2756)	16497(±3781)	9577(±1671)	2951.2(±236)	6130(±243)	2759(±1668)
	90 min	2129(±90)	400(±213)	nd	nd	1892(±1556)	nd	16651(±3002)	15464(±2455)	4886(±1713.2)	nd	4025(±122)	1093(±164)
	120 min	1561(±19)	nd	nd	nd	nd	nd	16950(±3701)	14586(±2366)	3979(±1166)	nd	2248(±3080)	nd
	150 min	1154(±176)	nd	nd	nd	nd	nd	14914(±3445)	14255(±2332)	nd	nd	973(±318)	nd

[a] nd, Not detectable, figures in brackets are S.D.

TRSII continued to form with increased duration and fermentation temperature. The higher the fermentation temperature, the more the formation of TRSII but the greater the degradation of both TRSI and theaflavins. The differences in the response between TRSI and TRSII demonstrated that not all thearubigins continued to form with extended fermentation. It is probable that the decline in theaflavin and possibly TRSI levels resulted in TRSII formation. It should also be ascertained whether the huge decline recorded for simple theaflavins (Fig. 2) was responsible for the equally significant increases in TRSII (Table 1).

Another aspect that arises from these results is the relation between liquor brightness and the chemical composition of black tea. A positive correlation is usually observed between liquor brightness and total theaflavin content of black tea, such that processing conditions which result in higher total theaflavin content tend to give brighter liquors and *vice versa*. In the present study, the total theaflavin content showed a positive quadratic trend over the experimental duration at the 20 °C fermentation temperature. However, liquor brightness, determined both by the spectrophotometric method and by taster, consistently declined with increased fermentation time, irrespective of rise in theaflavin levels such that the shorter the fermentation duration and the lower the fermentation temperature, the higher was the brightness. This could mean that another chemical group was responsible for the decline in liquor brightness as fermentation duration increased and that the formation of that chemical group was enhanced at elevated temperature. In this study, TRSII formation was predominant and increased more than TRSI with rise in fermentation duration and temperature. The same processing conditions are those in which the decline in all theaflavins was most pronounced. The results therefore demonstrated an inverse relationship between liquor brightness and theaflavin formation on the one hand, and TRSII formation on the other. The relationship between TRSII formation, theaflavin breakdown and loss of liquor brightness needs to be confirmed.

Briskness and/or astringency is usually associated with theaflavins, TFDG equivalents and the unoxidized catechins, especially the gallated catechins, ECG and EGCG (Biswas et al., 1973; Ding et al., 1992). In the present study, substantial amounts of these two catechins remained unoxidized. The residual levels of both catechins were dependent on both the temperature and duration of fermentation. At the 20 °C fermentation temperature, considerable quantities of unoxidized ECG and EGCG still remained in black teas after 120 min of fermentation. However, increasing the temperature to 30 °C led to a more rapid decline in the EGCG levels than ECG. The relative oxygen consumption between the two catechins was in conformity with their relative redox potentials (Bajaj et al., 1987). However, it was noted that the disappearance of the two catechins did not result in a corresponding increase in TFDG at the elevated fermentation temperature during the later stages of fermentation. Instead, TFDG levels declined. The taster's evaluation of briskness of tea liquors showed that the 20 °C fermentation temperature produced significantly more brisk teas than the 30 °C. Biswas et al. (1973) showed that it was total theaflavins, and ECG that accounted for the major part in the total variations in briskness of black tea. The present results showed that processing conditions which favoured less degradation of simple theaflavins, and the retention of higher residual ECG and EGCG levels produced more brisk tea liquors. Theaflavins and unoxidised catechins are thought to have considerable human health benefit (Apostolides & Weisberger, 1995; Cheng et al., 1986, 1991; Sano et al., 1991). Thus, aside from their influence on liquor astringency, these results highlight the possibility of manipulating processing conditions of duration and temperature to enhance the health-benefit potential of black tea. Maintaining a low fermentation temperature and short duration will ensure less conversion of ECG and EGCG but greater formation of the simple and dominant theaflavin. The resultant black teas are then brisk, and bright and probably offer more benefit to human health.

Acknowledgements

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