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Influence of fermentation time on the development of compounds responsible for quality in black tea

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Abstract

The compounds responsible for tea quality, such as theaflavins (TFs) and thearubigins were found to increase with fermentation time. However, TF reached a maximum, declining subsequently. The water extract of black tea decreased with fermentation time. Caffeine concentration remained unchanged. The digallate equivalent of theaflavin, colour index and briskness index were found to peak at the optimum fermentation time. Polyphenols declined more quickly during the initial stages, followed by a steadily declining trend. Fermentation time had little impact on the gallic acid concentration. Among the catechins, epigallocatechin oxidized fastest, followed by epigallocatechin gallate and epicatechin gallate.

Keywords: Tea; Fermentation; Theaflavins; Catechins

1. Introduction

Black tea is consumed throughout the world for its unique taste, briskness and flavour. Tea is the most widely consumed and cheapest non-alcoholic drink next to water. Black tea is manufactured from the tender leaves of *Camellia sinensis* (L) O Kuntze. Catechins are the major biochemical constituents (amounting to ca. 20% on dry weight basis) present in tea leaves and they get oxidized to form theaflavins (TFs) and thearubigins (TR) during fermentation (Hampton, 1992). Catechins and their oxidation products are mainly responsible for the taste and astringent character of black tea. Apart from quality characters, catechins are also found to possess properties of benefit to human health (Juni Terao, Sayuri Miyamoto, & Kaeko Murota, 2001; Weisburger, 2001; Yokozawa, Nakagawa, Shu, & Juneja, 2001). The major catechins present in tea leaves are catechin (C), epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCG). Their structures are given in Fig. 1. Fermentation is one of the important processes in black tea manufacture. During fermentation, the simple substrates, i.e., catechins, are acted upon by the oxidative enzymes, polyphenol oxidase and peroxidase, to form theaflavins and thearubigins (Lakshminarayanan & Ramaswamy, 1978). Theaflavins are composed of simple theaflavin (TF), theaflavin-3-gallate (TF-3-G), theaflavin-3′-gallate (TF-3′-G) and theaflavin-3,3′-digallate (TF-3,3′-DG) and are shown in Fig. 2.

The time, temperature, pH, relative humidity and oxygen availability during fermentation are the crucial factors responsible for the formation of high levels of desired products (Cloughley, 1980; Cloughley & Ellis, 1980; Obanda, Owuor, & Mangoka, 2001; Rajeev, Rajappan, & Balasubramanian, 2002). Of these, the time of fermentation is important, since both increase and decrease in fermentation time can lead to poor quality tea. Although some attempts had been made to show the effects of fermentation time on tea quality, the reports on the formation of

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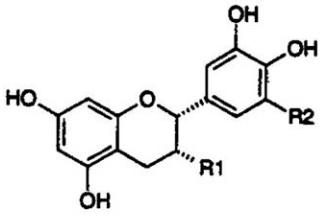
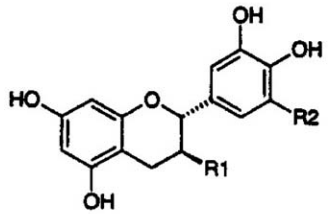
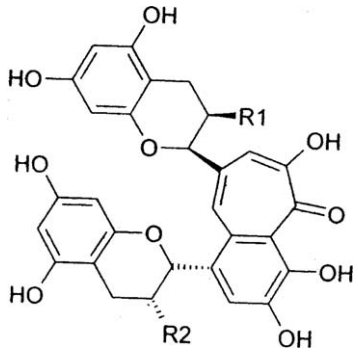


1. (-)-Epicatechin (EC)
   R1 = OH
   R2 = H
2. (-)-Epicatechin gallate (ECG)
   R1 = Galloyl
   R2 = H
3. (-)-Epigallocatechin (EGC)
   R1 = R2 = OH
4. (-)-Epigallocatechin gallate (EGCG)
   R1 = Galloyl
   R2 = OH



1. (+)-Catechin (C)
   R1 = OH
   R2 = H

Fig. 1. Structures of catechins present in tea leaves.



1. Simple theaflavin (TF)
   R1 = OH
   R2 = OH
2. Theaflavin-3-gallate (TF-3-G)
   R1 = Galloyl
   R2 = OH
3. Theaflavin-3'-gallate (TF-3'-G)
   R1 = OH
   R2 = Galloyl
4. Theaflavin-3,3'-digallate (TF-3,3'-DG)
   R1 = R2 = Galloyl

Fig. 2. Theaflavin fractions of black tea.

theaflavins and oxidation of catechins during fermentation are very limited (Bhatia, 1960; Owuor, Orchard, & McDowell, 1994). Also there appears to be no report on the oxidation pattern of individual catechins during fermentation. Hence, an attempt was made to study the effect of fermentation time on the oxidation of individual catechins and formation of theaflavins during the fermentation process of clonal black tea manufacture.

2. Materials and methods

Young tea shoots, comprising an apical bud and two expanded leaves of the clone UPASI-3, harvested from UPASI Tea Research Foundation Experimental Farm, were used for the study. Freshly plucked tea shoots were loaded in the withering trough at the rate of 10 kg/m^2. Ambient air was passed through the leaves for 16 h to bring about adequate physical and chemical withering. The withered leaves were passed through a mini CTC (crush, tear and curl) machine five times to get adequate maceration. The time at which the last cut ended was taken as zero time. The fermenting 'dhool' was drawn at regular time intervals, i.e., 15 min, up to 3 h, and analysed for theaflavins and total polyphenols, as suggested by Ullah (1977). The 'dhool' samples, drawn at 15 min intervals, were dried in a mini fluid-bed drier to a final moisture content of 3%. The black tea samples were sorted, using an 'Endecotts' sieve shaker, and the pekoe fannings grade was taken for analysis.

The black tea samples were analysed for quality parameters namely, theaflavins (TF), thearubigins (TR), highly polymerized substances (HPS), total liquor colour (TLC), digallate equivalent of theaflavin (DGETF), briskness index (BI) and colour index (CI) by following the method of Thanaraj and Seshadri (1990). The tea samples were also analysed for their water extract (WE) and caffeine (AOAC,

1995), theaflavin fractions (Bailey, McDowell, & Nursten, 1990) and catechin fractions (ISO, 1999). The experiment was repeated thrice and the results were statistically analysed using SPSS version 7.5.

3. Results and discussion

3.1. Fermentation time vs formation of theaflavins and oxidation of polyphenols

The changes taking place during fermentation of black tea are shown in Fig. 3. During fermentation, the polyphenols are oxidized to *o*-quinones (highly unstable form) by the enzyme polyphenol oxidase (PPO) and they interact to form TFs and TR (Hampton, 1992). The thearubigins, on reaction with TF and proteins, form complex highly polymerized substances (HPS). Theaflavins contribute toward the briskness and brightness of the tea liquor and TR are mainly responsible for colour and body of the liquor (Hilton & Ellis, 1972). Highly polymerized substances increase the colour of the brew. Total liquor colour (TLC) is the measure of brightness of the infusion. The formation of TFs increased with time during the early stages of fermentation. As time progressed, TF reach a maximum and then declined slowly. The time at which maximum TFs are formed was taken as the optimum fermentation time for the particular clone. The clone UPASI-3 is one of the fast fermenting clones and its optimum fermentation time is 45 min (Ramaswamy, 1978). The observations in the present study were in line with the earlier findings. Fig. 3 shows that the formation curve of TFs climbed steadily, reaching a maximum at 45 min of fermentation. However, after 110 min, there was little change in the level of TFs during fermentation. This could be due to changes in the activity of PPO.

The oxidation of polyphenols during fermentation followed a different trend. It was faster during the initial stages and the substrate concentration was found to decline steadily as fermentation proceeded. The activity of PPO declined with the fermentation time. This is due to the formation of complexes between oxidized polyphenols and the enzyme. The insoluble complexes increased with the oxidation of polyphenols. Rate of enzyme reaction declined at higher concentration of polyphenols, to maintain the maximum velocity of enzyme reaction (Takeo, 1965, 1966). This is the reason for the formation of more polymeric TR with fermentation time.

3.2. Fermentation time vs quality parameters of black tea

Quality parameters were analysed in the black tea and the results are presented in Table 1. The maximum theaflavin level did indeed occur in the tea manufactured at the 45th minute of fermentation. The levels of TR increased with the fermentation time up to 90 min and then tended to decline slightly. HPS and TLC followed much the same trend as TR. The TSS present in black tea were found to decline with time. The WE in tea had a direct relation with cuppage (Ramaswamy, Rajendiran, & Raju, 1993). From this result, it could be inferred that the increased fermentation time might lead to teas with poor cuppage. The level of caffeine was not affected by fermentation time. This might be due to the restriction of purine metabolism to the withering process alone.

The briskness and colour indices were worked out as suggested by Ramaswamy (1986) and are presented in

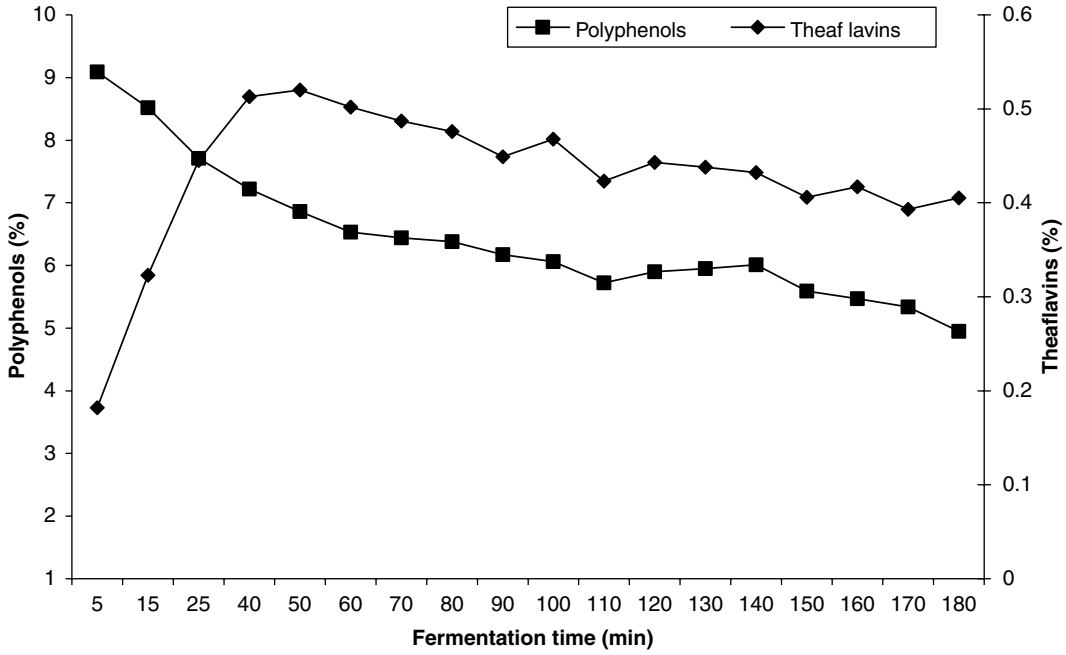


Fig. 3. Formation of theaflavins and oxidation of polyphenols during fermentation process of black tea manufacturing.

Table 1
Effect of fermentation time on the quality parameters of black tea

Time (min)	TFs (%)	TR (%)	HPS (%)	TLC	WE (%)	Caffeine (%)	DGETF (%)	BI	CI
15	1.61	10.5	10.0	1.87	44.7	2.71	0.70	37.3	7.86
30	2.29	12.5	12.7	2.79	42.6	2.93	1.10	43.9	9.08
45	2.59	12.7	14.0	3.13	41.2	2.90	1.31	47.2	9.79
60	2.43	13.1	15.1	3.28	40.7	2.74	1.27	47.0	8.62
75	2.38	13.3	15.6	3.41	40.6	2.88	1.26	45.3	8.24
90	2.29	14.2	17.2	3.55	40.0	2.86	1.22	44.6	7.30
105	2.28	13.1	16.9	3.54	40.2	2.68	1.23	46.0	7.63
120	2.20	12.9	17.5	3.53	39.9	2.81	1.19	44.1	7.24
135	2.03	13.2	18.0	3.60	39.6	2.86	1.12	41.7	6.54
150	1.98	12.8	18.3	3.64	39.5	2.51	1.10	44.2	6.39
165	2.01	12.8	18.2	3.60	39.5	2.76	1.13	42.3	6.53
180	1.96	13.3	18.2	3.81	39.0	2.72	1.09	42.2	6.23
CD at $P = 0.05$	0.14	1.15	0.70	0.20	0.71	0.35	0.07	3.35	0.72

BI, briskness index = (TFs × 100)/(TF + Caffeine). CI, colour index = (TFs × 100)/(TR + HPS).

Table 1. For better tea, the colour index should be between 5 and 11 in order to have the liquor balanced with colour and briskness. If the colour index value crosses 11, then the tea lacks colour, and, when it falls below five, the liquor will be coloured and flat with low briskness. In the present study, the colour index values for all the teas were between 6 and 10. The briskness index values showed that the tea manufactured at the 45th minute was the most brisk. The normal range of briskness index proposed for south Indian teas is 12.5–22.5. Brighter liquors will have a briskness index above 22.5. But, when it drops below 17.5, the liquors tend to have a harsh taste and when it exceeds 17.5 the liquor gets brisker (Ramaswamy, 1986). Briskness index values of the teas in the present study were all above 30.

3.3. Fermentation time vs theaflavin fractions

Apart from their anti-oxidant activities (Lai kwok Leung et al., 2001), theaflavin and its gallates are unique in their astringency and their relative distribution in black tea varies with fermentation time. Theaflavin digallate is 6.4 times more astringent than simple theaflavin and 2.88 times more astringent than theaflavin monogallates (Sanderson et al., 1976). Therefore, TF alone is not a good indicator of quality of black tea. Thanaraj and Seshadri (1990) introduced a new factor, termed digallate equivalent of theaflavin (DGETF), to express the astringency of black tea. The DGETF values correlated well with the price realization of Kenyan teas (Owuor & Obanda, 1995). The DGETF values for the teas manufactured at different time intervals are presented in Tables 1 and 2. The tea manufactured at the 45th minute was found to have the highest value. Simple theaflavins decreased whereas, the TF-3-G and TF-3,3′-DG increased as the fermentation time increased from 15 to 180 min of observation (Table 2). This shows that drying at the optimum fermentation time leads to a tea with good, astringent properties and hence good quality.

Table 2
Effect of fermentation time on the theaflavin fractions[a] of black tea

Time (min)	TF	TF-3-G	TF-3′-G	TF-3,3′-DG	DGETF (%)
15	39.0	22.8	21.0	17.2	0.70
30	30.6	27.4	19.8	22.2	1.10
45	27.7	29.1	18.5	24.7	1.31
60	24.9	29.6	18.7	26.8	1.27
75	24.4	29.1	19.0	27.5	1.26
90	23.8	30.0	18.3	27.9	1.22
105	22.8	30.0	19.2	28.1	1.23
120	22.8	30.3	18.7	28.3	1.19
135	22.2	30.3	17.1	30.5	1.12
150	21.6	29.9	17.9	30.7	1.10
165	21.1	28.9	19.1	30.9	1.12
180	20.4	30.0	19.2	30.4	1.09
CD at $P = 0.05$	0.44	0.18	0.08	0.342	0.07

[a] Expressed as percent of the sum of all theaflavins.

3.4. Fermentation time vs oxidation of catechins

The catechin fractions in the black teas manufactured at different times are given in Table 3. The catechins were found to be oxidized with fermentation time, though both catechin and gallic acid were almost unaffected. This may be due to the formation of free gallic acid and catechin from the other catechin fractions such as EGCG, ECG and EGC, by oxidative degallation. The liberation of free gallic acid was also observed by Coggon, Moss, Graham, and Sanderson (1973). Similar results were reported by Fernandez, Pablos, Martin, and Gon alez (2002) and Xie, von Bohlen, Klocken Kamper, Jian, and Gunther (1998). During fermentation, considerable quantities of EGCG, EGC and ECG were oxidized to form theaflavins and their gallates. The rate of oxidation follows the order EGC > EGCG > ECG. But none of the catechins were found to be exhausted completely, in contrast to the results of Obanda et al. (2001). All the catechins were detected in the black teas even after 3 h of fermentation, which may be due to inactivation of PPO through complex formation between it and oxidized flavanols.

Table 3
Oxidation of individual catechins as affected by the fermentation time

Time (min)	Gallic acid (%)	EGC (%)	C (%)	EC (%)	EGCG (%)	ECG (%)
15	0.087	0.778	0.632	0.310	8.15	0.574
30	0.081	1.93	0.207	0.923	4.11	0.377
45	0.074	1.71	0.240	0.914	3.23	0.289
60	0.069	1.59	0.271	0.844	2.83	0.231
75	0.060	1.34	0.250	0.787	1.77	0.165
90	0.067	1.02	0.321	0.417	2.03	0.144
105	0.057	0.849	0.304	0.750	1.21	0.098
120	0.060	0.630	0.295	0.614	1.46	0.084
135	0.062	0.623	0.341	0.870	1.63	0.084
150	0.048	0.365	0.249	0.636	1.42	0.055
165	0.052	0.448	0.252	0.434	1.13	0.054
180	0.051	0.351	0.237	0.867	1.25	0.052
CD at $P = 0.05$	0.012	0.180	0.133	0.219	0.164	0.022

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