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Effect of manufacturing techniques on individual flavanols and flavour profiles in CTC black tea under south Indian conditions

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Abstract: Changes in individual flavanol composition and flavour profiles were studied to optimize the condition for production in relation to tea quality. Individual catechins and its gallated products were found to decrease during processing. Theaflavin fractions were found to increase during initial stages of processing after which it was reduced during end of processing. Thearubigin fractions increased gradually from harvested fresh leaves till processing. Flavour profiles were carried out during different processing stages. A sharp increase in group I and II volatile flavour compounds (VFC) were noticed during withering and fermentation after which a decline in VFC was noticed. Group I compounds such as hexanal and 1-penten-3-ol were decreased during processing while trans-2-hexenal, cis-3-hexenol and 1-octen-3-ol increased. Group II compounds linalool, geraniol and methyl salicylate increased upon processing. Flavour index was found to be increased during processing and it suggests that most of the flavour compounds are formed during processing.

Keywords: theaflavins, linalool, geraniol, fermentation, trans-2-hexenal

1. Introduction

Tea is one of the principal food sources of flavonoids in the human diet because of its higher level of consumption combined with its relatively higher flavonoid content (Ho, Osawa, Huang, & Rosen, 1994).Flavonoids occurring in green tea are mainly flavanols, which constitutes about 90 % (w/w) of the phenolic compounds in the leaves (Lakenbrink, Lapczynski, Maiwald, & Engelhardt, 2000). The main tea phenolic compounds are catechins and their gallates and also referred as polyphenols (Harbowy & Balentine, 1997). Main catechin fractions are simple catechin (+C), Epigallo catechin (EGC), Epicatechin (EC), Epigallocatechin gallate (EGCG), Epicatechin gallate (ECG). These individual catechin fractions are grouped as gallated, non-gallated, trihydroxylated, dihydroxylated and catechin index. Flavonoids in black tea are mainly theaflavins and thearubigins which are formed by the oxidative and condensative transformation of flavanols during fermentation and their levels greatly

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correlate with quality and price levels of black tea. The major theaflavins in black tea are simple theaflavin (TF), theaflavin-3-gallate (TF-3-G), theaflavin-3'-gallate (TF-3'-G), theaflavin-3, 3'-di gallate (TF-3, 3'gallate) and digallate equivalent theaflavin (DGETF). Thearubigins are separated into two large groups i.e. Thearubigins I and Thearubigins II, due to differences in chemical properties. These compounds are mainly responsible for the taste and astringent character of black tea. Most acceptance of black tea is due to its exquisite aroma which consists of more than 700 compounds. Aroma compounds are separated into two group's viz., group I and group II. Group I compounds impart greenish odour and are derived from lipids and fatty acids. Group II compounds impart floral aroma, which are formed by break down of terpenoids, carotenes etc. Linalool, geraniol and benzaldehyde are the major components of group II compounds. The ratio of group II to group I is named as flavour Index (Obanda & Owuor, 1995).Black tea processing is generally classified in to four steps viz., withering, cutting, fermentation and drying. Withering is the first step of black tea manufacture, in which the loss of water in fresh shoots makes it suitable for subsequent processes. Cutting is the process, during which the leaves are macerated to facilitate the mix up of enzymes and their substrates. Fermentation is an oxidation process, during which the polyphenols are oxidized and condensed to form theaflavins and thearubigins. Drying reduces the moisture content to about 3 % and it was reported that drying influences the formation of many aroma compounds. Each and every processing step has a major influence on the final black tea quality because chemical and physical changes occurring in the crop shoots of tea in various stages. Also there appears to be no reports on the changing pattern of individual flavanols, flavour compounds during different stages of tea processing. Hence, an attempt was made to study the effect of different manufacturing techniques on the transformation of individual flavanols and flavour compounds during processing of clonal black tea manufacture.

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2. Materials and methods

2.1. Sampling and black tea manufacture

About 2 kg of green leaves comprising an apical bud and the terminal three leaves were collected and spread on the withering troughs at the rate of 3 kgs per square foot. Ambient air at room temperature was passed with the velocity of 45 cfm (cubic feet per minute) through the leaves for a period of 16 hours to bring about adequate physical and chemical wither. The withered leaves were passed through miniature CTC machine five times to get adequate maceration. The cut *dhool* was allowed to ferment in a chamber maintained at 25 °C and 95 percent relative humidity to their optimum fermentation period. The fermented *dhool* was dried in a fluid bed drier with a blast of hot air of inlet temperature 120 °C for 15 minutes, to get the tea with final moisture content of less than three percent. At the end of individual processing, the samples were drawn and inactivated at 103^0 C for seven minutes to terminate the enzyme activity. These samples were dried in hot air oven at 70^0 C for 24 h and dried samples were pulverized using pestle and mortar and sieved to obtain the fine powder which was used for the analysis of catechin, theaflavin and thearubigin fractions.

2.2. Analysis individual flavanols

The analysis of catechin fractions, theaflavin fraction, thearubigin fractions and total theaflavins were carried out by following the method of ISO 14502-2; 2005; Madanhire, 1995; Roberts & Smith, 1961 and Thanaraj & Seshadri, 1990. Individual catechin fractions were grouped as gallated, non-gallated, dihydroxylated and trihydroxylated catechins according to Owuor & Obanda, 2007. Constituents of gallated forms of catechins included EGCG and ECG while non gallated form of catechins was derived from the sum of EGC, +C and EC. Trihydroxylated catechins composed of EGC and EGCG whereas dihydroxylated catechins comprised of +C, EC and ECG.

2.3. Extraction and analysis of Volatile Flavour Compounds (VFC)

The samples drawn as fresh green leaf, withered leaf, cut *dhool*, fermented *dhool* and made tea at various stages of manufacturing were extracted with dichloromethane using simultaneous distillation extraction (SDE) apparatus

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(Yamanishi, Botheju, & Jayanthi de Silva, 1989). About 25 g of the above samples were placed in single neck round bottom flask (1000 mL) and covered with the boiled distilled water (250 mL) at 70^0 C. This was placed in heating mantle (100^0 C) and linked to one arm of the SDE apparatus, whilst a small flask (250 mL) which contained 50 mL dichloromethane at 40^0 C (in a thermo stated water bath) was simultaneously connected to the other arm of the apparatus. Along with the above connections, 10 mL of dichloromethane was added into the U-tube located in the middle of the apparatus. Distillation was limited to 1h. The condensate of dichloromethane was dried with anhydrous sodium sulphate and a small aliquot of this concentrate was used for VFC analysis.

The analysis was carried out using a Gas chromatograph (Perkin Elmer Auto system XL) using ethyl caproate as internal standard. About 1 µL of the extract was injected and Flame ionization Detector (FID) was used for detection. The temperature of the injector and FID were 200^0 C and 250^0 C respectively. A 60 m X 0.25 mm i.d of HP-INNOWAX capillary column was used. The initial temperature of the column was 50^0 C for 2 min and increased at the rate of 5^0 C per min to 180^0 C, thereafter maintained at the same temperature (180^0 C) for 15 min. Nitrogen was employed as the carrier gas with a flow rate of 1 mL per min. The compounds of the sample were identified in comparison to their GC retention times with those of authentic standards (Sigma). The identified flavours were expressed in terms of percentage of their relative distribution with respect to ethyl caproate.

2.4. Statistical analysis

The experiments were repeated thrice and the results were statistically analyzed using SPSS version 11.5.

3. Results and discussion

3.1. Distribution of catechin fraction during processing

Reduction of catechin fractions (EGC, +C, EC, EGCG and ECG) during different stages of processing was observed coinciding with oxidation (Table 1). Since the gallated, non-gallated, dihydroxylated and trihydroxylated catechins were derived from the catechin fractions, they also followed the same trend as was observed with individual molecules. Reduction in catechin fractions and

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total catechins during withering was supported by Bokuchava & Skobeleva, 1980 who reported that all catechin fractions were found to be decreased except epicatechin. A significant reducing trend was observed during fermentation stage where all the enzymes and substrates are brought together and a complex reaction proceeds. These findings are supported by the work of Collier et al. (1973), reported that the oxidation of EGCG with EC produced TF-3-Gallate, while oxidation of EGCG with ECG produced Theaflavin digallate. Yao & Nursten, (1997) also found that chemical oxidation of EGCG alone yielded polyphenolic profiles. There was a little change in level of catechin fractions after fermentation. The grouped catechins also registered same trend as that of individual catechins.

3.2. Effect of processing stages on theaflavin fractions

From the table 2, it is evident that TF fractions followed identical pattern in their relative distribution where TF-3-3'-gallate retained major proportions followed by TF-3-gallate, TF-3'-gallate and simple theaflavins. An exponential enhancement in the levels of simple TF, TF-3-gallate TF-3'-gallate and TF-3-3'-gallate was observed from fresh leaves till fermentation process. However theaflavin fractions were reduced marginally due to drying of fermented *dhool*. On the other hand, linear increase in total TF and DGETF was evident. Considering each processing stages individually, TF fractions were formed more during fermentation. Formation of TF-fractions could be due to the oxidation of catechins and their gallates present in the green leaves, in which simple TF are formed by the oxidation of EC and EGC (Takeo & Oosawa, 1976). Collier et al. (1973), reported that TF-3-G gallate is the oxidative product of EGCG and EC and he also informed that increase in the levels of TF-3'-G could be due to degradation of ECG and EGC. The formation of TF-3, 3'-G could be due to the oxidative damage of ECG and EGCG (Coxon, Holmes, Ollis, & Vora, 1970). The reduction in the level of TF-fractions during drying could be due to deactivation of enzymes by high temperature. During fermentation higher amount of TF-fractions were formed which could be due to catechins which get reduced by oxidation in presence of an enzyme polyphenol oxidase and

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peroxidase which leads to the formation of TF constituents.

3.3. Effect of processing stages on thearubigin fractions

While analyzing the total TR, a gradual increase was observed from harvested fresh leaves to the processing stages (Table 3). Incremental difference between each stage of processing and cultivars showed marked variations. Among the TR fractions, TR-II is the most predominant one followed by TR-I by their distribution. Unlike the TF fractions, development of TR pigments is unique and increased right from harvesting and its retention in made tea is significant. Based on differences in chemical properties of thearubigins Roberts & Smith in the year 1961 established that it could separate into two larger groups, i.e TRSI and TRSII. The level of total TR increased during withering; these findings correlated well with the observation made by Obanda, Owuor, Mang'oka, & Kavoi, (2004). But the individual TR i.e) TRSI and TRSII increased during withering, these results of which were already reported by Obanda et al. 2004. TRSI was found to decrease while TRSII increased. But he informed that both TRSI and TRS II were found to increase during fermentation and here again the same results were observed. This could be due to the oxidation and condensation of theaflavins. The formation of TR fractions during drying could be due to the degradation of gallated flavanols with increased temperature which could be incorporated into thearubigins (Temple, Temple, van Boxtel, & Clifford, 2001).

3.4. Percentage relative distribution of individual flavours during processing

During withering, the group I volatile flavour compounds, hexenal, 1-penten–3–ol, trans- 2-hexenal were decreased while 1-penten-3-ol, cis-3-hexenol and 1-pentanol increased (Table 4). In the case of group – II compounds, all compounds showed an increasing trend during withering thereby increasing the value of flavour index. Prior to cutting, withered leaves recorded an increase in the amount of 2-hexanol, trans-2-hexenol and 1-octen-3-ol. Majority of the group II compounds were increased during cutting except phenyl ethanol, cis-nerolidol and methyl jasmonate. A slight increase in the trans-2-hexenol, 2-hexanol, cis-3-hexenol and 1-octen-

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3-ol was observed during fermentation while group II compounds showed identical trend as that of cutting. During drying, marginal improvement in the alpha ionone, beta-ionone, cis-nerolidol and methyl jasmonate was documented and rest of the aroma compounds deteriorated.

Aroma is an important quality parameter of black tea and it had a significant relationship with flavour index value and sensory evaluation (Owuor, 1992). Reduction in the levels of group-I compounds during withering was studied by Owuor, Wanyiera, Njeru, Munavu, & Bhatt, (1989), reported that lipid degradation during withering decrease the unsaturated fatty acids, which undergo region- and enantio-selective oxidative cleavage to form aroma compounds. Takeo, 1981 found that vigorous catechin oxidation during processing of black tea from fresh leaf tissue results in the depression of cis-3-hexenol and its esters with the consequent increase in the amount of trans-2-hexenal. The level of group –II compounds were increased during withering and fermentation thereby increasing the flavour index, which was supported by the observation of Hazarika & Mahanta, (1983) who reported that the degradation of carotenoids increases the flavour constituents. The flavour index value was found to be increased during drying by the degradation of carotenoids by the reaction of pyrolytic.

4. Conclusion

The present study has clearly established that the amount of individual flavanols and flavours were greatly altered by different processing techniques. Most of compounds responsible for the quality, taste and aroma were formed only after harvested during the processing stages. In conclusion, the analysis of individual catechins, theaflavins, thearubigins and volatile flavour constituents during different stages of processing could be used in the optimization of manufacturing conditions and to control, preserve the quality of black teas produced in south India.

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Table: 1: Distribution of catechin fractions during processing

Compounds	Fresh leaf	Withered leaf	Cut *dhool*	Fermen--ted *dhool*	Made Tea	SE m±	CD @P=0.05
EGC %	5.75	4.88	3.86	2.88	2.60	0.03	0.07
+C %	0.89	0.84	0.80	0.71	0.68	0.02	0.04
EC %	2.36	1.67	1.63	1.05	0.82	0.02	0.04
EGCG %	8.20	7.52	7.06	5.56	3.80	0.02	0.05
ECG %	1.80	1.48	1.35	1.02	0.98	0.02	0.06
GC %	10.00	9.00	8.41	6.58	4.78	0.03	0.08
NGC %	9.00	7.39	6.29	4.64	4.10	0.02	0.04
DHC %	5.05	4.03	3.83	2.98	2.65	0.03	0.06
THC %	13.95	12.40	10.92	8.44	6.40	0.05	0.10
CI	0.30	0.25	0.27	0.25	0.28	0.01	0.03

EGC – epigallocatechin; +C- simple catechin; EC-epicatechin; EGCG- epigallocatechin gallate; ECG- epicatechin gallate; GC-gallated catechin; NGC- non gallated catechin; DHC-dihydroxylated catechin; THC- trihydroxylated catechin; CI- catechin index

Table 2: Effect of processing on Theaflavin fractions (% relative distribution)

Processing Stages	Simple TF	TF-3-gallate	TF-3'-gallate	TF-3, 3'-gallate	TF%	DGETF %
Fresh leaf	0.57	6.23	5.02	11.32	0.24	0.04
Withered leaf	0.68	7.12	6.36	13.03	0.35	0.07
Cut *dhool*	3.36	12.39	10.24	17.58	0.57	0.16
Fermented *dhool*	10.17	35.69	28.35	49.38	0.82	0.65
Made Tea	9.32	27.54	22.56	42.12	1.12	0.74
SE m±	0.02	0.03	0.04	0.05	0.02	0.01
CD @P=0.05	0.04	0.06	0.09	0.12	0.04	0.02

Table

DGETF (%) = Total TF (Simple TF/6.4+TF- monogallates/2.22+TFDG)/100

6.4 and 2.22 refers the astringencey threshold levels of TF-3, 3' gallate and TF-3- gallate ; Simple TF – simple theaflavin; TF-3-gallate – theaflavin-3-gallate; TF-3'-gallate – theaflavin-3'-gallate; TF-3,3'-gallate – theaflavin-3, 3'-gallate; TF – theaflavins; DGETF – digallate equivalent of theaflavins

Table 3: Effect of processing on Thearubigin fractions

Processing Stages	TR-I (%)	TR-II (%)	Total TR (%)
Fresh leaf	1.07	2.37	3.44
Withered leaf	1.12	3.58	4.70
Cut *dhool*	1.61	5.64	7.25
Fermented *dhool*	2.18	7.18	9.36
Made Tea	2.45	7.69	10.14
SE m±	0.10	0.15	0.21
CD @P=0.05	0.23	0.36	0.41

TR-I – thearubigin fraction I

TR-II – thearubigin fraction -II

Total TR – total thearubigins

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4: Individual flavours during processing (Percentage relative Distribution with that of internal standard)

Compound	FL	WL	CD	FD	MT
Group-I					
Hexenal	2.86	2.15	2.02	1.95	1.02
1-penten -3- ol	1.14	0.94	0.79	0.69	0.43
1-pentanol	1.35	1.62	1.23	0.95	0.59
2-hexanol	1.16	1.89	2.83	3.21	1.32
Trans - 2 -hexenal	2.21	1.77	2.58	3.36	2.21
6-methyl -5- -hepten -2- one	1.35	2.65	2.01	1.85	0.74
Cis-3-hexenol	1.68	1.99	1.63	2.23	1.29
1-Octen -3-ol	1.04	1.36	1.98	2.18	1.65
Group – II					
Alpha pinene	0.41	0.67	0.84	0.99	0.52
Linalool oxide - I	0.84	1.12	1.83	1.97	0.65
Linalool oxide - II	0.91	1.19	1.72	1.95	1.09
Citronellal	0.17	0.34	0.41	0.79	0.26
Benzaldehyde	0.18	0.21	0.52	0.76	0.12
Linalool	2.84	4.54	5.23	6.29	5.26
Phenyl acetaldehyde	0.61	0.87	1.26	1.69	2.16
Geranyl acetate	0.03	0.36	0.32	0.31	0.21
Methyl salicylate	0.75	1.08	1.36	1.58	0.85
Nerol	0.29	0.54	1.08	1.79	1.41
Geraniol	1.31	1.51	1.84	1.87	1.06
Alpha - ionone	0.41	0.56	0.79	0.97	1.15
Benzyl alcohol	0.21	0.35	0.61	0.72	0.32
Phenyl ethanol	0.36	0.49	0.35	0.31	0.12
Beta - ionone	0.21	0.45	0.61	0.72	0.92
Cis - nerolidol	0.28	0.46	0.38	0.30	0.49
Methyl jasmonate	0.02	0.08	0.06	0.06	0.22
Sum of group – I	12.79	14.37	15.07	16.42	9.25
Sum of group - II	9.83	14.82	19.21	23.07	16.81
Flavour Index	0.77±0.05	1.03±0.08	1.27±0.06	1.40±0.06	1.82±0.07

FL – fresh leaf; WL-withered leaf; CD-cut *dhool*; FD-fermented *dhool* ; MT - made tea

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