

Fingerprint of black teas from India: identification of the regio-specific characteristics

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Abstract

In order to assess the intrinsic characteristics of black teas from different regions of India, a chemical and biochemical fingerprint was generated. Relevant constituents of black teas were analysed, using standard physico-chemical, spectrophotometric, HPLC and GC-MS analytical methodologies, to carry out the fingerprinting. Palampur teas had the lowest theaflavins (TFs) and total colour (TC) and high caffeine, total soluble solids (TSS) and viscosity. Teas from Nilgiris, Rosekandy and Kurti had the highest TFs and TC. Assam teas had moderate TFs and TC. Nilgiris and Annamalai teas had low caffeine, TSS and viscosities. Assam, Rosekandy and Kurti teas had high caffeine, moderate TSS and viscosities. Twenty-five volatile flavour compounds (VFCs) were identified by GC-MS as fingerprint markers. Only Palampur teas had nonanal but no dihydroactinidiolide. Annamalai tea had the lowest *n*-hexanol and Nilgiri-Wynad tea had the lowest geranyl acetate. Only Kurti tea contained xylene. This fingerprint will help to trace the origin of teas. © 2002 Elsevier Science Ltd. All rights reserved.

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1. Introduction

India is a leading tea producer, consumer and exporter. India produced 840 million kg of tea worth Rs.6300 crores in the year 2000, out of which south India contributed 24% and north India contributed 76%. In the same year, India exported 200 million kg of tea worth Rs.1800 crores. The world production of Crush–Tear–Curl (CTC) tea was 1194 million kg, and orthodox tea production was 839 million kg during 1999 (Ramadurai, 2000). Black tea, manufactured from young tender shoots of *Camellia sinensis* (L.) O. Kuntze, is the most widely consumed non-alcoholic drink and its flavour quality and taste have been shown to change with variations in geographical (Takeo & Mahanta, 1983; Yamanishi, Kobayashi, Uchida, & Mori, 1968) and climatic (Cloughley, Ellis, Pelnington, & Humphrey, 1982; Howard, 1978) conditions. Yamanishi et al. (1968) compared flavours of teas from different parts of the world, while Cloughley et al. (1982) compared flavours of teas in different seasons in Malawi, Africa.

Indian teas, especially from Darjeeling, Assam and Nilgiris (Trinitea) are valued for their characteristic aroma and taste. These teas are much sought after and used by consumers throughout the world (Bala Subramaniam, 1995). Many reports are available about antioxidant and anticancer properties and health benefits of tea (Dreosti, Wargovich, & Yang, 1997; Tijburg, Mattern, Folts, Weisgerber, & Katan, 1997; Wiseman, Balentine, & Frei, 1997; Yang & Wang, 1993; Yang, Liao, Kim, Yurkow, & Yang, 1998).

The important chemical constituents which influence the taste and flavour in tea brew are polyphenols, caffeine, sugars, organic acids, volatile flavour compounds and amino acids. Phenolic compounds of tea, such as theaflavins and thearubigins, are very important from an intrinsic quality point of view. These are responsible for the colour, flavour and brightness of tea. Caffeine is responsible for the briskness. The physicochemical parameters, such as TSS and viscosity of the brew and bulk density (packed/loose) of the tea are important quality indicators. The volatile flavour compounds of tea and their variation in composition due to geographical and other process variables are of paramount importance from a quality point of view. Though

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considerable work has been done (Gulati & Ravindranath, 1996; Gulati, Tamang, & Ravindranath, 1999; Ullah, 1985) on the quality aspects of tea, a comparative study covering Indian regions has not so far been done. In view of the complexity of tea quality, a study has been carried out to fingerprint the Indian black teas obtained from different regions. The data generated, based on the studies, will help to trace the origin of teas.

2. Materials and methods

2.1. Samples

Samples of made tea (season—1999) were collected from different producing regions (Palampur, Assam, Nilgiris, Annamalai, Dibrugarh, and Darjeeling) with the help of regional offices of the Tea Board (India) and the United Planters Association of Southern India (UPASI) Valparai, in Tamil Nadu, and Institute of Himalayan Bioresource Technology (IHBT), Palampur in Himachal Pradesh. The samples obtained were stored in air-tight PET bottles at ambient temperature (25 ± 5 °C) and used for the fingerprint studies.

2.2. Chemicals and standards

All the solvents and chemicals used were of analytical grade and obtained from BDH (caffeine), Sigma-Aldrich, USA (Cumene), and Merck, Mumbai (Disodium hydrogen phosphate-anhydrous, ethyl acetate, methanol, oxalic acid, chloroform, acetonitrile).

2.3. Analysis for physicochemical parameters

2.3.1. Moisture

Moisture was determined according to AOAC (2000, Chap. 30, p. 12), and expressed as percentage loss in weight on drying in a hot air oven.

2.3.2. Bulk density

Bulk density (packed bulk density and loose bulk density) of black tea samples was carried out as per method of Ramaswamy (1995) described below.

- a. Loose bulk density: Tea samples filled in a glass cylinder using a hopper suspended 3 cm away from it. Filled it upto 500 ml level. Weighed the tea accurately. The weight of tea per unit volume (g/ml) is loose bulk density.
- b. Compacted or packed bulk density: Tea sample filled in a glass cylinder using a hopper suspended 3 cm away from it upto 500 ml level. The filled cylinder was tapped five times using soft rubber tubing. The weight and volume of tea at that time was noted. Weight of tea per unit

volume is the compacted or packed bulk density (g/ml).

2.3.3. Soluble solids

Totals soluble solids (TSS) of tea brew was determined (Hanan, Salmah, Azizah, & Russly, 2001), after sample preparation using a refractometer. Tea (2 g) was added to boiling distilled water (140 ml) in a clean beaker and allowed to boil (infuse) for 4 min. The brew was filtered through Whatman No. 5 filter paper, using a Buchner funnel, by applying a vacuum.

The refractometer was calibrated (Atago, Japan) using distilled water. The brew was well mixed and a drop of cooled brew placed on the prism of the refractometer. Illumination and colour compensation and temperature (20 °C, by circulating water through the provided channel, next to the prism) were adjusted. Readings were taken in triplicate and an average taken as TSS (%).

2.3.4. Viscosity

Brew, prepared as above, in a beaker (30 ml) was placed in the viscometer (Rheology international, Japan). A suitable spindle, keeping the RPM constant, was selected by trial and error. Readings were taken in triplicate and an average taken as the final viscosity of the tea brew.

2.4. Analysis for volatile flavour compounds (VFCs)

2.4.1. Extraction

Simultaneous distillation and solvent extraction (SDE), using a Likens–Nickerson apparatus (1964) for isolation of volatiles, was carried out. Black tea sample (50 g) was added to a 2 l round-bottom flask containing 1 l distilled water, along with 0.2 ml of internal standard (15 µl of cumene in 10 ml of pet. ether). Into another flask (200 ml), 25 ml of petroleum ether (40–50 °C fraction), 0.25 ml of ethanol and 25 ml of diethyl ether with magnetic bit were placed. These two R.B. flasks were attached to two arms of the Likens–Nickerson apparatus and extracted for 2.5 h. After extraction the solvent containing VFCs was evaporated on a water bath to 0.5 ml volume, which was then transferred to a test tube and stored at 4 °C for GC–MS analysis.

2.4.2. GC-MS analysis for VFCs

A Shimadzu GC-17A equipped with QP-5000 (Quadrupole) mass spectrometer was used. A fused silica capillary column SPB TM-1, coated with polydimethylsiloxane of 30 m length and 0.32 mm internal diameter and film thickness 0.25 µm, was used. Helium was the carrier gas with a flow rate of 1 ml/min. Split ratio was 1:50 and ionisation voltage was 70 eV. The injection port temperature and detector port temperature were maintained at 220 °C. Oven temperature

programme was: 40 °C (3)—2 °C/min, 100 °C—4 °C/min, 220 °C (7); a sample of 1 µl was injected for each analysis. Identification of compounds was achieved by comparison mass spectra (NIST library and Adams, 1989) and Kovats indices (Davies, 1990; Jennings & Shibamoto, 1980).

2.5. Spectrophotometric analysis for theaflavins (TFs) and thearubigins (TRs)

Biochemical assessment of black tea quality was done from estimation of TFs and TRs of tea brew. In the present studies, a rapid procedure for estimating theaflavins and thearubigins of black tea was adopted (Ullah, 1986). The absorbances were measured on a UV-visible spectrometer, Cintra 10 (Australia).

The tea sample (9 g) added to 375 ml of boiling water in a conical flask and the boiling continued for 10 min using an air condenser on a water bath. The tea infusion was filtered through cotton cloth and cooled to room temperature.

The infusion (6 ml) was mixed with 6 ml of 1% (w/v) aqueous solution of anhydrous disodium hydrogen phosphate and the mixture extracted with 10 ml of ethyl acetate by quick repeated inversion for 1 min. The separated bottom layer drained, remaining was the ethyl acetate layer (the TF fraction) and diluted with 5 ml ethyl acetate. Optical densities, E_1 , E_2 , E_3 ; were obtained on extracts prepared as follows:

10 ml of TF extract were diluted to 25 ml with methanol (E_1);

to 1 ml of infusion 9 ml of water were added and made up to 25 ml with methanol (E_2);

to 1 ml of infusion was added 1 ml of aqueous oxalic acid (10% w/v), and 8 ml of water and made up to 25 ml with methanol (E_3). Optical densities of E_1 , E_2 , and E_3 were measured at 380 and 460 nm.

At 380 nm % TF = $2.25 \times E_1$
 % TR = $7.06 (4 E_3 - E_1)$
 At 460 nm Total colour = $6.25 \times 4 E_2$

$$\% \text{ Brightness} = \frac{E_1}{4E_2} \times 100$$

2.6. HPLC analysis for caffeine

2.6.1. Chromatographic system

The determination of caffeine in tea samples was carried out by an improved HPLC method (Pura Naik & Nagalakshmi, 1997). A Shimadzu HPLC solvent delivery system controller (Model LC-6A), with system controller (Model SCL-6A), was used. The injection system used a 20 µl loop. Detection was by a UV-visible spectrophotometer (SPD-6AV) set at a sensitivity of 0.08

AUFS and a wavelength of 276 nm. A µ-Bondapak C₁₈ column (3.9 mm × 15 cm) was used. The data processor used was CR-4A chromatopac. The mobile phase used was acetonitrile and triple distilled water (20:80 v/v) at a flow rate of 1 ml/min.

2.6.2. Standard caffeine curve

Standard stock solution was prepared by dissolving 80 mg caffeine in 100 ml water. Working standard was prepared by diluting 10 ml of stock solution to 100 ml to give a concentration of 0.08 µg/µl.

Working standard solution (5–25 µl) was injected on the HPLC, and peak area responses were obtained. A standard graph for caffeine was prepared by plotting concentration versus area.

2.6.3. Sample preparation

Two grams of black tea were weighed into a 250 ml beaker and 177 ml of boiling water were added, brewed for 6 min on a boiling water bath (80 °C), filtered through Whatman No.44 filter paper, and 2 ml filtrate were subjected to analysis for caffeine content.

2.6.4. Separation of soluble caffeine

A Sep-pak C₁₈ cartridge was activated by first passing through 2 ml of methanol by means of a glass syringe, and flushing to expel any remaining methanol.

Tea extract (brew) (2 ml) was then passed through the cartridge and eluate was rejected. Again air was flushed through to expel any remaining water. Caffeine was eluted from the cartridge with 6 ml of chloroform (drop by drop), followed by air, into an evaporating flask.

The chloroform was removed on a water bath under vacuum. The residue in the flask was dissolved in water and made up to 4 ml. An aliquot (5–10 µl) of this solution was injected into the HPLC.

2.6.5. Clean up of Sep-pak C₁₈ cartridge for re-use

The used cartridge was cleaned up with 35% (v/v) methanol in water (5 ml) followed by 80% (v/v) methanol in water (4 ml) and finally with 2 ml of methanol for re-use. The cartridge could be re-used 25 times.

The percent caffeine was calculated by comparing the peak area responses with the standard caffeine curve (calibration curve).

3. Results and discussion

The results of the physico chemical characterisation in terms of caffeine, TSS, viscosity, moisture and bulk density are presented in Table 1. Most of the teas from North Indian regions/gardens have high (3.4–3.9%) soluble caffeine contents. This may cause the high briskness of these teas. The teas from south Indian regions/gardens have comparatively less (2.0–2.8%)

Table 1
Physico-chemical characteristics of black tea

Sample name	Soluble caffeine (%)	TSS (%)	Viscosity (mPa)	Moisture (%)	Bulk density (g/cm ³)		Theaflavin, TF (%)	Thearubigin, TR (%)	Brightness (%)	Total colour
					Loose	Packed				
Palampur G ₁	3.69±0.005	1.0±0.001	11.0±0.018	3.4±0.002	0.27±0.013	0.35±0.005	0.18±0.020	9.48±0.250	7.41±1.00	1.78±0.180
Palampur G ₂	3.72±0.012	1.0±0.002	10.2±0.009	2.8±0.010	0.20±0.015	0.27±0.019	0.14±0.006	13.5±0.501	5.23±1.04	1.56±0.046
Assam BOP	3.92±0.004	0.8±0.002	8.1±0.006	5.6±0.013	0.35±0.001	0.42±0.002	0.45±0.015	8.57±0.860	8.53±1.20	2.30±0.148
Assam OP	2.84±0.005	0.6±0.005	5.6±0.020	5.8±0.021	0.24±0.003	0.31±0.011	0.27±0.006	14.6±0.530	2.52±0.361	3.94±0.221
Nilgiris high grown CTC-BOP	2.08±0.023	0.4±0.010	6.3±0.013	6.4±0.018	0.40±0.005	0.46±0.006	0.81±0.006	14.8±0.119	16.3±0.648	3.85±0.127
Nilgiris Wynaad CTC-BOP	2.03±0.011	0.4±0.012	6.2±0.011	6.0±0.006	0.41±0.002	0.47±0.015	0.75±0.038	12.7±0.212	16.6±0.733	3.60±0.132
Annamalai	2.78±0.018	0.4±0.009	4.8±0.002	4.6±0.011	0.43±0.010	0.49±0.004	0.64±0.012	11.8±0.108	10.2±0.925	3.49±0.021
Rosekandy	3.46±0.006	0.6±0.013	8.5±0.016	6.4±0.002	0.36±0.012	0.41±0.014	0.88±0.006	12.5±0.217	16.6±0.181	4.85±0.097
Kurti	3.54±0.002	0.6±0.019	6.8±0.005	6.4±0.005	0.39±0.001	0.43±0.006	0.82±0.002	13.8±0.015	14.0±0.005	4.82±0.020

Values expressed are mean±S.D. of five experiments.

soluble caffeine content. This observation confirms the findings of Pura Naik and Nagalakshmi (1997) for south Indian teas. TSS of brew were measured at cup level to indicate the solids content present in a cup of brew, which is ready to consume. This method (Hanan et al., 2001) is different from earlier methods (ISI, 1973; AOAC, 2000, Chap. 30, p. 12). In the present method, it was found that the Palampur teas have the highest TSS and viscosities, followed by Assam teas, Kurti and Rosekandy tea. South Indian teas have lower TSS and viscosities. This is the first report of TSS at cup level and viscosity of Indian teas.

Ramaswamy (1995) carried out studies on free flow or uncompact (loose) bulk density and compacted (tapped/packed) bulk density determination of CTC black teas by designing an instrument. He found that variable falling height, diameter of funnel and L/D ratio of receptacle all have some bearing on density of tea and the free flow (loose) bulk density of BP grade was lowest (0.39–0.43 g/cm³) compared to RD grade (0.50–0.53 g/cm³) and SFD grade (0.54–0.56 g/cm³)

The results of the bulk density (packed/loose) in the present study show a definite trend. Palampur (G₁ and G₂) and Assam OP (Orange Pekoe) teas have the lowest bulk densities. Assam BOP, Rosekandy and Kurti teas have slightly higher bulk densities. Nilgiris (High grown/Wynaad CTC-BOP) teas and Annamalai teas have the highest bulk densities (packed/loose). This is attributed to the grades and granular size of the teas. In the present study, the loose bulk density of Nilgiris (High grown/Wynaad CTC-BOP) teas is comparable with the findings of Ramaswamy (1995) for BP grade. However, this is the first report of these values from other regions.

The golden yellow TFs and the reddish-brown TRs, produced as a result of enzymatic oxidation of catechins and their subsequent condensation during black tea manufacture, largely determine liquor characteristics of black tea (Balentine, Wiseman, & Bouwns, 1997; Finger, Kuhr, & Engelhardt, 1992; Ullah, Gogoi, & Baruah,

1984). The results of the fingerprint, in terms of TFs, TRs, total colour and brightness (Ullah, 1986) are presented in Table 1. TF content of the tea samples varied from 0.14 to 0.88%, TR content from 8.57 to 14.8%, total colour from 1.56 to 4.85, and brightness from 2.52 to 16.61%.

The Palampur (G₁ and G₂) teas had the lowest TF and total colour and low brightness and high TR. Assam OP tea had slightly high TF, TR and total colour but the lowest brightness of all. Among the north Indian BOP teas, Assam BOP had the lowest TF, TR and total colour and a high brightness. The South Indian teas (Nilgiris high grown/Wynaad-CTC) had high TF, TR, total colour and brightness. The North Indian teas (Rosekandy and Kurti) had the highest TF, TR, total colour and brightness.

The TF, TR values, reported by Ullah (1986) for Assam teas and by Ranganath, Marimuthu, and Raju (1994), are not comparable to the values reported in the present study. However, the samples analysed in the study are procured from factory samples, whereas the samples studied by earlier groups were prepared at pilot scale or on a miniature scale. This may be the reason for variation.

The variability in these constituents may be attributed to factors such as origin of the material, crop husbandry, plucking standard, method of processing, grades, packaging, storage and, very importantly, the genotype.

Results of the fingerprint in terms of volatile flavour compounds (VFCs) are presented in Tables 2 and 3. Twenty-five compounds are identified and taken as markers. Their quantities were calculated with reference to internal standard in different tea samples. The teas from different origins can be marked by the composition of VFCs identified. Gulati and Ravindranath (1996) explained seasonal variation of the VFCs in Kangra teas. Gulati et al. (1999) studied the aroma profiles with respect to clonal variations in Kangra teas. However, they could identify 10 VFCs. In the case of

Table 2
Identification of major volatile flavour compounds from black tea

Sample No.	Compound	KI cal	M ⁺	m/z
1	<i>n</i> -Hexanal	772	100	41,55,42,69,57
2	<i>E</i> -2-hexenal	825	98	41,42,55,69,57
3	<i>Z</i> -3-Hexenol	842	100	41,67,82,55,69
4	2-hexenol	848	100	57,82,41,43,44
5	<i>n</i> -Hexanol	853	102	56,43,41,55,42
6	<i>n</i> -Heptanal	875	114	43,41,70,44,55
7	Cumene	911	120	105,120,77,51,79
8	Benzaldehyde	939	106	77,106,105,51,50
9	(<i>E,Z</i>)-2,4-Heptadienal	975	110	81,41,53,67,110
10	(<i>E,E</i>)-2,4-Heptadienal	984	110	81,41,53,67,110
11	Nonanal	1006	142	41,57,43,55,56
12	Phenyl acetaldehyde	1017	120	91,65,92,120,51
13	Benzyl alcohol	1022	108	79,108,77,107,1
14	<i>cis</i> -Linalooloxide	1058	170	59,43,55,94,68
15	<i>trans</i> -Linalooloxide	1073	170	59,43,55,94,68
16	Linalool	1085	154	71,41,93,55,43
17	Phenyl ethyl alcohol	1097	122	91,92,65,122,51
18	α -Terpineol	1173	154	59,43,93,81,121
19	Methyl salicylate	1180	152	120,92,152,121,65
20	<i>cis</i> -Geraniol	1232	154	41,69,93,67,53
21	Indole	1291	117	117,89,90,63,118
22	Geranyl acetate	1358	196	41,43,69,68,67
23	β -Ionone	1455	192	177,43,41,44,178
24	Dihydroactinidiolide	1471	180	111,43,109,137,67
25	Nerolidol	1518	222	41,69,43,93,71
26	Phytol	2010	296	71,43,57,69,123

Palampur teas, nonanal is present whereas dihydroactinidiolide is absent. In the present study we could identify more compounds and found nonanal as the characteristic compound for this region since it is absent in teas from other regions. Phytol is also absent in Palampur teas, whereas it is present in considerable quantities in teas from the remaining regions. Palampur teas contain more volatiles. Ullah (1985) studied aroma constituents, of Assam and China hybrid teas and their manifestation during tea processing. He identified eight aroma constituents, concluding that aroma constituents of tea are mainly inherent in the leaf and their manifestation is largely governed by black tea processing methods. China hybrid tea has, in general, a higher concentration of VFCs than Assam-type tea. In the present study, we found that Assam OP tea does not contain any *trans/cis*-2-hexenol, *n*-hexanol or *n*-heptanal, while Assam BOP has a lower leaf alcohol (*Z*-3-hexenol) but almost five times the leaf aldehyde (*E*-2-hexenal) of Assam OP. Annamalai tea has the lowest *n*-hexanol. Ranganath et al. (1994) studied effects of withering processes and found that the sum of VFC I (consists mainly of C₆ aldehydes and alcohols which are lipid degradation products and impart inferior quality to tea) was the highest and sum of VFC II (dominated by terpenoid compounds contributing sweet aroma of the

Table 3
Analysis of black tea for volatile flavour compounds (VFCs; $\mu\text{g}/100\text{ g}$)

Sample No.	Volatile flavour compounds	Palampur G1	Palampur G2	Assam OP	Assam BOP	Anamalai	Nil-HG CTC	Nil-Waynad	Rosekandy	Kurti ^a
1	<i>n</i> -Hexanal	A	A	T	168	35	199	A	128	31
2	Leaf aldehyde (<i>E</i> -2-hexenal)	3871	2581	434	1959	889	1230	1581	2071	1349
3	Leaf alcohol (<i>Z</i> -3-hexenol)	2781	1890	94	12	18	63	51	84	97
4	<i>Trans/cis</i> -2-hexenol	272	244	A	A	A	43	A	48	102
5	<i>n</i> -Hexanol	565	503	A	43	15	50	79	44	A
6	<i>n</i> -Heptanal	147	234	A	A	55	47	A	55	A
7	Benzaldehyde	125	165	104	38	35	88	62	31	39
8	(<i>E,Z</i>)-2,4-Heptadienal	170	323	A	A	5	A	8	T	T
9	(<i>E,E</i>)-2,4-Heptadienal	275	381	7	T	9	A	14	11	11
10	Nonanal	114	129	A	A	A	A	A	A	A
11	Phenyl acetaldehyde	147	133	382	378	355	99	350	797	1072
12	Benzyl alcohol	806	754	37	24	31	31	T	T	T
13	<i>cis</i> -linalool oxide	719	503	31	24	28	20	22	15	34
14	<i>Trans</i> -Linalool oxide	1745	1287	79	55	89	60	89	55	105
15	Linalool	1313	851	19	13	256	96	438	A	45
16	Phenyl ethyl alcohol	210	511	365	133	256		408	122	200
17	Alfa-terpineol	455	285	36	41	26	17	36	10	10
18	Methyl salicylate	1495	1257	36	34	25	47	41	40	95
19	<i>cis</i> -Geraniol	822	657	18	9	74	34	149	20	15
20	Indole	T	T	23	13	10	8	11	9	10
21	Geranyl acetate	181	201	19	9	8	A	7	A	A
22	β -Ionone	47	105	33	18	52	6	44	14	33
23	Dihydroactinidiolide	A	A	68	26	22	7	43	10	24
24	Nerolidol	453	245	27	19	19	A	29	14	26
25	Phytol	A	A	A	493	443	256	888	589	1308

A = absent, T = trace. Values expressed are mean of three experiments.

^a Special marker xylene is present upto 1400 $\mu\text{g}/100\text{ g}$ tea.

tea) was the lowest for teas from the freeze-withering process and higher in the case of natural/artificial-withered teas than unwithered ones. They could identify sixteen VFCs. In the present studies we found that Nilgiri high grown CTC tea had no nerolidol while Nilgiris-Wynaad tea had lowest geranyl acetate content. Rosekandy tea does not contain any linalool, while it is present in all other tea samples. Only Kurti tea had xylene, while this is absent in other samples. In conclusion it can be said that every tea has its own VFC marker, and no two samples match. This will therefore aid in tracing the origin of tea samples.

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