*Asian Journal of Chemistry* Vol. 21, No. 9 (2009), 6803-6810

# Phenolic Profiles and Antioxidant Properties of Turkish Black Tea Manufactured with Orthodox Method

BULENT KARADENIZ and ILKAY KOCA*
*Department of Food Engineering, Faculty of Engineering,*
*Ondokuz Mayis University, Samsun, Turkey*
*E-mail: itosun@omu.edu.tr*

In this research, it was aimed to determine the amount of catechins and polymerized catechins in and antioxidant activity of Turkish teas manufactured with Orthodox method. Tea samples were obtained from 20 tea plants which belong to the Turkish Tea Board (Çay-Kur, Turkey). The samples were analyzed for dry matter, antioxidant activity as ferric reducing antioxidant power (FRAP), total phenolics, theaflavin (TF), thearubigin (TR). Catechins and caffeine amounts were also determined by high performance liquid chromatography (HPLC). According to the results of this study, dry matter ranged between 91.26-92.97 %; FRAP value between 788-1079.82 μmol $g^{-1}$; total phenolics between 72.90-83.88 mg $g^{-1}$; TF value between 0.12-0.38 %; TR value between 11.64-13.80 %. Besides, gallocatechin (GC) was in the range of 0.36-0.89 %, catechin (C) 0.02-0.36 %, epigallocatechin gallat (EGCg) 0.02-0.15 %, epicatechin (EC) 0.02-0.81 %, epicatechin gallat (ECg) 0.01-0.21 %. Caffeine content was found to be between 2.13 and 2.54 %.

**Key Words: Black tea, Caffeine, Catechins, Ferric reducing antioxidant power.**

## INTRODUCTION

Tea is grown in about 30 countries and is the most widely consumed beverage in the world, after water. Depending on the manufacturing process, teas are classified into three major types: non-fermented green tea (produced by drying and steaming the fresh leaves and thus, no fermentation, *i.e.*, oxidation, occurs); semi-fermented oolong tea (produced when the fresh leaves are subjected to a partial fermentation stage before drying) and fermented black and red (pu-erh) teas (which undergo a full fermentation stage before drying and steaming, although the fermentation of black tea is oxidation and that of pu-erh tea is attained using microorganisms)[1]. Black tea represents *ca.* 80 % of tea products[2]. Turkey is the sixth largest world producer of black tea after China, India, Sri Lanka, Kenya and Indonesia[3]. Turkey is also one of the most black tea consumed countries.

The manufacturing of black tea is characterized by a high degree of enzymatically catalyzed aerobic oxidation of the leaf polyphenols followed by a series of chemical condensations[4]. In black tea, during the fermentation process most of the catechins, forming the main flavanols of green tea are oxidized and polymerized to theaflavins and thearubigins, which are responsible for the colour, flavour and brightness of tea[3].

Recently, catechins have received considerable attention both from the scientific community and the general public because of their claimed health benefits and functionally such as antioxigenicity, antimutagenicity, antitumorgenicity and anticarcinogenicity[5].

The objectives of this study are (1) to determine theaflavin and thearubigin levels of the tea extracts; (2) to identify individual catechins of the samples; (3) to determine the antioxidant activities of the extracts.

## EXPERIMENTAL

Pure standards of epicatechin (EC), gallocatechin (GC), epicatechin gallat (ECg) and epigallocatechin gallat (EGCg) were purchased from Sigma Chemical Co. (USA). Catechin hydrate (C) was obtained from Fluka Chemie (Switzerland) while caffeine was purchased from Carlo Erba (French). Methanol and acetic acid were purchased from J.T. Baker (Holland). 2,4,6-Tripyridyl-s-triazine (TPTZ) was obtained from Acros Organics (New Jersey, USA). All solvents were of analytic or HPLC grade.

Black tea samples used in this research were obtained from 20 tea plants which belong to the Turkish Tea Board (Çay-Kur, Turkey). Samples were collected at the first plucking period (the beginning, middle and the end) as three replicates. Samples obtained from the 1st, 2nd, 3rd, 4th, 5th and 6th sieve from each plant were mixed in equal proportions and milled to pass through a 2 mm sieve.

### Analytical determinations

**Dry matter:** A known weight of each sample was dried in an oven for at least 16 h at 105 °C, then cooled in a desiccator and re-weighed[6]. The percentage dry matter in a sample was then calculated.

**Antioxidant activity:** The sample (1 g) was extracted with hot water 100 mL for 15 min. The tea solution was allowed to cool at room temperature. Then, the tea solution was filtered. After appropriate dilution, the solution was mixed with ferric-TPTZ reagent (prepared by mixing 300 mM acetate buffer, pH 3.6, 10 mM 2,4,6-tripyridyl-s-triazine in 40 mM HCl and 20 mM $FeCl_3$ in the ratio of 10:1:1) and measured at 593 nm. $FeSO_4$ was used as a standard and the antioxidant activity expressed as μmol $g^{-1}$ FRAP[7].

**Total phenolics:** The amount of total phenolics was determined using Folin-Ciocalteu reagent, as described by Singleton and Rossi[8]. Results were expressed as milligram gallic acid equivalents (GAE) per gram.

**Theaflavin and thearubigin analysis:** Theaflavin and thearubigin analysis were done according to the procedure described by Ullah[9]. Three grams of tea samples was stirred with hot water 125 mL at 96 ºC for 10 min. After filtration, the tea solution was allowed to cool at room temperature. A portion of the tea solution (20 mL) was mixed with 20 mL of 1 % aqueous solution anhydrous disodium hydrogen phosphate. The mixture was extracted with 20 mL ethyl acetate in a separating funnel and shaken for 10 min. Part of the ethyl acetate layer (2 mL) was

diluted to 25 mL with methanol. The absorption of this methanol solution was recorded as $A_1$.

Part of the tea solution (1 mL) was mixed with aqeous oxalic acid (10 %, w/v) (1 mL) and distilled water (8 mL) and then diluted to 25 mL with methanol. The absorption of this methanol solution was recorded as $A_2$. Absorbances of $A_1$ and $A_2$ were measured with a UV-visible spectophotometer (Jasco V-530, Japan) at 380 nm, with methanol as a blank. Theaflavin (TF) and thearubigin (TR) were calculated according to the following equations:

$$\% \text{ TF} = 2.25 \times 2A_1 \quad (1)$$

$$\% \text{ TR} = 7.06 \times (4A_2\text{-}2A_1) \quad (2)$$

**Catechins and caffeine analysis:** Tea extracts were prepared according to the procedure described by Khokhar *et al.*[10]. One gram of black tea samples was extracted with 100 mL boiled distilled water for 5 min. After the extraction, the pH was adjusted to 3.2 with citric acid and the extract was filtered through a 0.45 μm filter (Biocrom MN 718020, Phonex nylon filter 25 mm). After appropriate dilution, aliquots were taken and analyzed by high performance liquid chromatography.

High performance liquid chromatography separation and identification were carried out according to the procedure described by Ding *et al.*[11]. 30 % Aqueous methanol containing 0.1 % acetic acid was used as eluent with a flow rate of 1.0 mL $min^{-1}$. Detection was carried out at 270 nm. Samples were analyzed using a Thermoquest (USA) HPLC system equipped with a model P100 pump, an SN4000 controller unite, a model AS 2000 autosampler and a model UV 1000 UV-Vis detector. Separation was carried out using a 4.6 mm × 250 mm Luna 5u $C_{18}$ column. The IBM (300 GL) computer was used to evaluate the peak area. The area and the retention time of the chromatographic peaks were compared with pure standards (C, EC, GC, ECg, EGCg and caffeine). Standard solutions were injected into the HPLC and peak area responses were obtained. A standard graph for each component was prepared by plotting concentration *versus* area. Quantification was carried out from integrated peak areas of the sample and corresponding standard graph.

## RESULTS AND DISCUSSION

The results of dry matter, total phenolics, polymerized catechins and antioxidant activity of black tea samples are given in Table-1.

**Dry matter:** The content of dry matter in black teas varied between 91.26 and 92.97 %. The results of present study were compared with the dry matter contents of Turkish black tea determined by Yilmaz[12]. Present findings were found to be close to the reported values (88.7-92.9 %).

Moisture content of tea is important for its quality and storage. Increase in the moisture content over a period of time resulted in the deterioration of quality constituents of tea during storage. Tea, being hygroscopic, is more susceptible to lipid hydrolysis, auto oxidative reactions and enzymatic browning during storage. Reactions were found to be accelerated by moisture[13]. Moisture content of tea changes

TABLE-1
DRY MATTER, TOTAL PHENOLICS, THEAFLAVIN, THEARUBIGIN AND FRAP VALUES OF THE SAMPLES

| Sample | Dry matter (%) | FRAP (μmol $g^{-1}$) | Total phenolics (mg $g^{-1}$) | Theaflavin (%) | Thearubigin (%) |
|---|---|---|---|---|---|
| 1 | 92.29 ± 0.59 | 874.90 ± 14.84 | 78.02 ± 0.19 | 0.24 ± 0.01 | 12.66 ± 1.64 |
| 2 | 92.03 ± 0.48 | 824.95 ± 16.83 | 72.90 ± 0.18 | 0.22 ± 0.01 | 13.04 ± 0.59 |
| 3 | 92.11 ± 0.36 | 909.77 ± 4.14 | 78.70 ± 0.26 | 0.17 ± 0.07 | 12.08 ± 0.55 |
| 4 | 91.54 ± 0.54 | 913.31 ± 80.07 | 79.14 ± 1.70 | 0.28 ± 0.03 | 12.01 ± 0.43 |
| 5 | 91.33 ± 0.23 | 905.21 ± 46.19 | 79.72 ± 0.88 | 0.23 ± 0.01 | 13.80 ± 0.71 |
| 6 | 91.85 ± 0.54 | 812.64 ± 17.04 | 72.94 ± 1.13 | 0.30 ± 0.08 | 12.01 ± 0.98 |
| 7 | 91.39 ± 0.28 | 1079.82 ± 48.76 | 76.54 ± 0.91 | 0.38 ± 0.01 | 12.41 ± 1.43 |
| 8 | 91.90 ± 0.23 | 967.08 ± 49.12 | 75.80 ± 0.73 | 0.25 ± 0.01 | 12.31 ± 0.54 |
| 9 | 91.61 ± 0.31 | 1069.26 ± 30.46 | 80.44 ± 0.46 | 0.21 ± 0.04 | 12.05 ± 0.64 |
| 10 | 92.24 ± 0.48 | 788.95 ± 8.90 | 72.90 ± 0.29 | 0.23 ± 0.02 | 12.71 ± 0.73 |
| 11 | 92.64 ± 0.22 | 938.21 ± 108.31 | 75.74 ± 0.67 | 0.16 ± 0.05 | 12.65 ± 1.20 |
| 12 | 92.28 ± 0.02 | 1029.46 ± 51.85 | 75.96 ± 0.40 | 0.36 ± 0.03 | 13.12 ± 0.40 |
| 13 | 91.49 ± 0.39 | 1000.97 ± 5.09 | 73.80 ± 0.24 | 0.12 ± 0.05 | 13.05 ± 0.15 |
| 14 | 91.26 ± 0.10 | 895.54 ± 24.61 | 78.68 ± 1.59 | 0.36 ± 0.05 | 12.61 ± 0.48 |
| 15 | 91.70 ± 0.26 | 853.03 ± 23.01 | 73.74 ± 1.56 | 0.19 ± 0.01 | 13.34 ± 0.22 |
| 16 | 91.63 ± 0.54 | 954.69 ± 13.16 | 81.28 ± 1.55 | 0.23 ± 0.03 | 13.66 ± 0.81 |
| 17 | 91.90 ± 0.25 | 1018.23 ± 21.46 | 78.06 ± 0.53 | 0.34 ± 0.03 | 12.63 ± 0.19 |
| 18 | 92.97 ± 1.04 | 1023.36 ± 25.97 | 80.78 ± 0.38 | 0.27 ± 0.04 | 13.80 ± 0.42 |
| 19 | 91.77 ± 0.13 | 916.74 ± 35.98 | 79.90 ± 1.18 | 0.27 ± 0.01 | 11.64 ± 0.40 |
| 20 | 92.14 ± 0.72 | 952.69 ± 57.36 | 83.88 ± 2.01 | 0.27 ± 0.07 | 12.46 ± 0.51 |

according to drying and storage conditions. The moisture content at which tea can be stored without any risk of deterioration is 3-4 %[14]. It is reported that deteriorations occur at moisture contents higher than 10 %, however the critical limit is 13 % due to the presence of phenolic compounds[15].

**Antioxidant activity:** Antioxidant activity of the teas ranged between 788.95 and 1079.82 μmol $g^{-1}$. Antioxidant activity values of the samples determined in present study were higher than that Benzie and Szeto[16], who found antioxidant activity of 9 different black tea samples as 132-654 μmol/g in dry weight. Benzie and Strain[17] determined antioxidant power in black tea infusions as 500-900 μmol in 1 % infusion. These results are in accordance with present findings.

Tea has been considered a medicine and a healthful beverage since ancient times, but recently it has received a great deal of attention because tea polyphenols are strong antioxidants. The aqueous extract of the major tea polyphenols possesses antimutagenic, antidiabetic, antibacterial, antiinflammatory and hypocholesterolemic qualities. Among all tea polyphenols, especially catechins and gallic acid have been considered to be the main players in these beneficial effects on the human health[1]. Green tea is rich in catechins. Epigallocatechin gallat makes up about 40 % of the total catechin content and is widely accepted as the major antioxidant ingredient in green tea[18].

Ding *et al.*[11] checked oxygen-radical absorbing capacity of Assam green tea and Assam black tea. They found the oxygen radical-absorbing capacity of Assam green tea and Assam black tea. They stated that the antioxidative activity of black tea would be due not only to catechins but also to TFs and TR. Zeyuan *et al.*[19] also found that the antioxidative capacity of black tea was higher than that of green tea *in vivo*.

**Total phenolics:** The content of total phenolics in the teas varied between 72.90 and 83.88 mg $g^{-1}$. The values reported by several researchers were within the range of 1.8 to 134.9 mg/g[20,21]. Present values are within the range of the reported values.

Black tea consumption is associated with reduced risk of cardiovascular disease and several cancers. These beneficial effects have been ascribed to the marked antioxidant potential of polyphenolic compounds in the tea. These are primarily flavan-3-ols (catechins), flavonols, theaflavins and thearubigins[22].

**Theaflavin and thearubigin:** Characteristic pigments of black tea consist of two major groups, theaflavins and thearubigins, which are formed by enzymatic oxidation of four major green tea flavan-3-ols [(-) epicatechin, (-) epigallocatechin and their 3-O-gallates] during tea fermentation[23]. Theaflavins are astringent compounds that contribute importantly to the colour and taste of black tea beverage. During black tea manufacture, most of the catechin mass is converted to a less well defined group of compounds known as thearubigin (TR)[24].

Theaflavin value ranged between 0.12 and 0.38 % and thearubigin (TR) value was between 11.64 and 13.80 % (Table-1). The black tea samples had very high TF/TR ratio, which ranged from 1/32.7 and 1/108.7. Low TF and high TR contents in the samples may be due to over-fermenting. The TF content was lower than the values (0.21-0.94 %) reported by several researchers in Turkish black tea. Lakenbrink *et al.*[25] found that the content of TF was 1.17-1.5 % in black tea. Yao *et al.*[26] determined phenolic compounds in Australian teas and, reported the amount of TF to be 0.32-1.10 % in black tea leaf. Present results were lower than the values determined by Lakenbrink *et al.*[25] and Yao *et al.*[26]. Thearubigin (TR) content in black tea was reported as 9.45-20 % by several researchers[12,15,25]. Generally, these values are in aggrement with present findings. Yao *et al.*[26] found TR as 3.91-10.7 % in black tea. These values are lower than present results.

Theaflavin (TF), thearubigin (TR) and TF/TR are very important quality indicators of black teas. Black tea continues to ferment (involving further oxidation of TF) just following the completion of whole tea manufacturing, which is so-called post-fermentation or post-mature process. In fact, oxidation of phenolic compounds occurs in all types of teas during the storage period. Therefore, it is always recommended that fresh black teas should normally have a higher content of TF (TF > 1 %), a higher content of TR (TR > 10 %) and an adequate TF/TR ratio (> 0.1)[26].

**Catechins:** A wide variation in the content of catechins is observed among the black tea samples. Catechins and caffeine contents of the tea samples are given in Table-2.

TABLE-2
CATECHINS AND CAFFEINE CONTENTS OF THE SAMPLES (%)

| Samples | GC | C | EC | ECg | EGCg | Caffeine |
|---|---|---|---|---|---|---|
| 1 | 0.75 ± 0.08 | 0.26 ± 0.11 | 0.34 ± 0.17 | 0.16 ± 0.02 | 0.08 ± 0.02 | 2.27 ± 0.10 |
| 2 | 0.67 ± 0.08 | 0.14 ± 0.03 | 0.17 ± 0.10 | 0.12 ± 0.05 | 0.07 ± 0.02 | 2.28 ± 0.01 |
| 3 | 0.77 ± 0.12 | 0.21 ± 0.04 | 0.16 ± 0.10 | 0.19 ± 0.07 | 0.07 ± 0.04 | 2.30 ± 0.27 |
| 4 | 0.89 ± 0.12 | 0.28 ± 0.09 | 0.14 ± 0.05 | 0.11 ± 0.10 | 0.12 ± 0.06 | 2.54 ± 0.31 |
| 5 | 0.64 ± 0.06 | 0.17 ± 0.05 | 0.18 ± 0.07 | 0.17 ± 0.04 | 0.05 ± 0.03 | 2.22 ± 0.03 |
| 6 | 0.67 ± 0.15 | 0.17 ± 0.12 | 0.22 ± 0.11 | 0.14 ± 0.05 | 0.06 ± 0.06 | 2.25 ± 0.06 |
| 7 | 0.76 ± 0.01 | 0.23 ± 0.17 | 0.18 ± 0.06 | 0.14 ± 0.09 | 0.05 ± 0.01 | 2.52 ± 0.36 |
| 8 | 0.36 ± 0.03 | 0.02 ± 0.01 | 0.02 ± 0.00 | 0.15 ± 0.00 | 0.02 ± 0.01 | 2.33 ± 0.02 |
| 9 | 0.59 ± 0.14 | 0.11 ± 0.07 | 0.02 ± 0.00 | 0.13 ± 0.05 | 0.02 ± 0.00 | 2.36 ± 0.13 |
| 10 | 0.44 ± 0.03 | 0.16 ± 0.08 | 0.15 ± 0.12 | 0.01 ± 0.01 | 0.02 ± 0.03 | 2.29 ± 0.03 |
| 11 | 0.70 ± 0.02 | 0.22 ± 0.04 | 0.37 ± 0.01 | 0.15 ± 0.05 | 0.06 ± 0.06 | 2.26 ± 0.02 |
| 12 | 0.82 ± 0.03 | 0.28 ± 0.02 | 0.23 ± 0.06 | 0.12 ± 0.05 | 0.11 ± 0.02 | 2.49 ± 0.15 |
| 13 | 0.66 ± 0.01 | 0.23 ± 0.08 | 0.21 ± 0.08 | 0.21 ± 0.07 | 0.15 ± 0.02 | 2.26 ± 0.02 |
| 14 | 0.83 ± 0.05 | 0.27 ± 0.02 | 0.16 ± 0.02 | 0.12 ± 0.03 | 0.07 ± 0.06 | 2.33 ± 0.04 |
| 15 | 0.46 ± 0.22 | 0.08 ± 0.01 | 0.03 ± 0.01 | 0.10 ± 0.02 | 0.01 ± 0.02 | 2.27 ± 0.04 |
| 16 | 0.68 ± 0.04 | 0.21 ± 0.14 | 0.81 ± 0.02 | 0.14 ± 0.04 | 0.14 ± 0.04 | 2.27 ± 0.19 |
| 17 | 0.79 ± 0.07 | 0.31 ± 0.04 | 0.12 ± 0.02 | 0.11 ± 0.02 | 0.06 ± 0.01 | 2.13 ± 0.05 |
| 18 | 0.59 ± 0.44 | 0.36 ± 0.15 | 0.10 ± 0.08 | 0.16 ± 0.06 | 0.05 ± 0.05 | 2.41 ± 0.08 |
| 19 | 0.59 ± 0.06 | 0.12 ± 0.03 | 0.04 ± 0.01 | 0.14 ± 0.04 | 0.02 ± 0.03 | 2.33 ± 0.14 |
| 20 | 0.89 ± 0.12 | 0.32 ± 0.12 | 0.02 ± 0.01 | 0.10 ± 0.00 | 0.02 ± 0.00 | 2.45 ± 0.05 |

The levels of catechins in the black teas varied from 0.43 to 2.42 %. Gallocatechin (GC) was the most abundant flavan-3-ol for all the samples. Levels for GC ranged between 0.36 and 0.89 %. The second most abundant flavan-3-ol was EC, which ranged between 0.02 and 0.81 %. Catechin (C) content varied from 0.02 to 0.36 %. ECg content was found between 0.01 and 0.21 %. Epigallocatechin gallat (EGCg) was in the range of 0.02-0.15 % EGCg, which is the major catechin in tea leaves (*Camellia sinensis* L.) and which has been reported to exert various biological effects such as antioxidative, antimutagenic/anticarcinogenic and antibacterial. Tea catechins are degraded markedly during the black tea manufacturing process[27].

Lin *et al.*[4] analyzed polyphenol compunds in 10 different types of commercial tea, including unfermented, semifermented and fermented tea. They reported the following results for black tea: total catechins, 3.2 %; EC, 0.07 %; ECg, 0.4 %; EGCg, 1.3 %. Khokhar and Magnusdottir[21] used HPLC with acetonitrile as the eluent to measure the content of 5 catechins and caffeine in 4 black, 3 green and 6 fruit teas consumed in the U.K. They found the total catechin content ranged from 8.1 to 47.5 mg/g in black teas. The values for EC, EGCg and ECg in black tea were 1.4-5.6, 2.7-25.2 and 2.1-8.9 mg/g, respectively. Fernández *et al.*[28] used HPLC with water/acetonitrile/formic acid as the mobile phase to determine catechin profiles of 27 black teas originating from China, Kenya, India and Sri Lanka. They reported the following results for black tea samples: C, 0.070-0.466 %; EC, 0.010-1.735 %;

ECg, 0.186-1.811 %; EGCg, 0.338-8.233 %. Cabrera *et al.*[1], using an HPLC method with a photodiode array detector, measured 4 catechins and caffeine levels in 15 black tea sold in Spain. These authors reported that the EC content was between 4.0 and 11.4 mg/g, the ECg content between 4.4 and 20.6 mg/g and the EGCg content between 12.3 and 85.1 mg/g in black tea. Friedman *et al.*[29] determined catechins, theaflavins and alkaloids in 77 commercial black, green, specialty (brown rice, white, oolong) and herbal teas in the United States. They reported that the total catechin content ranged from 5.4 to 69.5 mg/g, the catechin (C) levels from 0.5 to 8.1 mg/g, the EC levels from 0.4 to 6.3 mg/g, the EGCg content from 0.5 to 28.4 mg/g and the ECg content from 1.7 to 26.8 mg/g in black tea. Present findings were generally different from all results.

**Caffeine:** The caffeine content of black teas varied from 2.13 to 2.54 %. The caffeine contents of the samples were lower than the values (2.68-5.4 %) reported by several researchers[4,11,12,28]. Present results were generally similar to the values determined in some tea samples, such as 1.84-5.18 %[15], 1.43-2.28 %[30], 2.21-3.97 %[31]. The stimulating effect of tea beverage is due to the presence of purine bases (caffeine, theobromine and theophylline). Caffeine is the major alkaloid of tea and it increases during the withering stage of black tea production[32]. It decreases during the fermentation and drying stages[31].

## Conclusion

Tea is the most popular beverage after water throughout the world. Tea consumption may provide health benefits, due to its high antioxidant activity and polyphenolic contents. The average intake of black tea is about 6.2 g/day/person in Turkey[33]. Since polyphenolic material in black teas is about 20-30 % of the dry solids in tea extract, the total polyphenolic ingestion in humans is therefore in the range of 1.2-1.8 g/day/person. This dose is much greater than the total RDA of vitamins C, E and β-carotene that is 70 mg/day and thus tea phenols are considered to be a large source of dietary antioxidants as reported by Vinson and Dabbagh[34].

According to the results obtained in this study, there were differences among the teas from different plants, with regard to phenolic profiles and antioxidant activity of the samples. These differences may be due to several reasons including species, age of the leaf (plucking position), climate, horticultural practices and processing conditions as well as degree of fermentation as reported by several researchers[4,21].

## ACKNOWLEDGEMENTS

This work is a part of Bulent Karadeniz's M.Sc. Thesis. The authors thank Ondokuz Mayis University Scientific Research Projects for financial support (Project no MF 083) and Turkish General Directorate of Tea Establishments (CAY-KUR) for providing black teas.

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(*Received*: 20 September 2008; *Accepted*: 30 July 2009) AJC-7697