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Nutritional and Functional Characteristics of Seven Grades of Black Tea Produced in Turkey

Arda Serpen,[†] Ebru Pelvan,[§] Cesarettin Alasalvar,*[,§] Burçe Ataç Mogol,[†] Havvana Tuba Yavuz,[#] Vural Gökmen,[†] Nihat Özcan,[§] and Beraat Özçelik[#]

[†]Department of Food Engineering, Hacettepe University, Beytepe-Ankara, Turkey

[§]TÜBİTAK Marmara Research Center, Food Institute, Gebze-Kocaeli, Turkey

[#]Department of Food Engineering, Istanbul Technical University, Maslak-Istanbul, Turkey

ABSTRACT: Seven grades of black tea [high-quality black tea (grades 1−3) and low-quality black tea (grades 4−7)], processed by ÇAYKUR Tea Processing Plant (Rize, Turkey), were examined for their proximate composition, dietary fiber, minerals, and water-soluble vitamins as well as total phenolic content, various antioxidant assays, phenolics (flavanols, alkoloids, condensed phenolics, and phenolic acids), chlorophylls, and carotenoids. Some variations, albeit to different extents, were observed ($p < 0.05$) among these parameters in seven grades of black tea. With respect to proximate composition, dietary fiber was the predominant compound (ranging from 49.68 to 54.31 g/100 g), followed by protein, carbohydrate, and, to a lesser extent, ash, moisture, and fat. Thirteen minerals, four water-soluble vitamins, six flavanols, two alkoloids, three condensed phenolics, one phenolic acid, two chlorophylls, and two carotenoids were identified in the seven grades of black tea. Total phenol content ranged from 7.52 to 8.29 g of gallic acid equivalents (GAE)/100 g, being lowest in grade 6 and highest in grade 1. With regard to antioxidant activities, a large variation in oxygen radical absorbance capacity (ORAC) values was observed among all grades of black tea (ranging from 777 μmol of trolox equivalents (TE)/g in grade 7 to 1210 μmol of TE/g in grade 3). The present work suggests that high- and low-quality black teas should not be distinguished on the basis of their nutritional and functional characteristics. The combination of nutritional compounds together with functional characteristics renders combination effects that provide the characteristic quality of each grade of black tea.

KEYWORDS: *grades, black tea, minerals, water-soluble vitamins, phenolics, catechin, antioxidant activity, chlorophylls, carotenoids*

INTRODUCTION

Tea is one of the most popular beverages in the world and is ranked at a level of being the second nonalcoholic drink after water. The world's tea production in 2009 was around 3,883,842 MT. China is the world's largest producer of tea, contributing 35.4% to the total global production, followed by India (20.6%), Kenya (8.1%), Sri Lanka (7.5%), Turkey (5.1%), Vietnam (4.8%), and Indonesia (4.1%). Other countries contribute 14.4% to the total global production. Turkey is the fifth largest producer of tea, with a production of 198,601 MT.[1]

Black tea is known to consist of considerable amounts of bioactives and phytochemicals apart from some nutritional compounds present in limited quantities. Tea is the only analyzed beverage to contain epigallocatechin gallate in quantifiable amounts. Epigallocatechin gallate and epicatechin gallate are the most abundant forms, each contributing 27% to the total catechin content (22.2 mg/100 mL) of black tea. Three flavonols (quercetin, kaempferol, and myricetin) are also reported in tea.[2] Black tea encompasses a range of polyphenols (e.g., flavanols, flavonol glycosides, and phenolics), oxidized dimers (e.g., theaflavins), and complex condensed tannins (e.g., thearubigins).[3,4] Oxidation of flavanols, leading to the formation of theaflavins and thearubigins responsible for the formation of the characteristic color and flavor of fermented tea, is catalyzed by catechol oxidase.[5] Therefore, control of the fermentation process has important effects on the flavor and color of tea, which depend on the degree of oxidation of tea phenolics. Theaflavins are yellow-orange or yellowish-brown in color and contribute to the astringency as well as another flavor characteristic of black tea known as "briskness";[6,7] on the other hand, thearubigins are black-brown or reddish-brown in color and contribute to the color, acidity, body, ashiness, and slight astringency of tea.[3,7]

Tea intake is associated with reduced risk of cardiovascular disease (CVD). Effects of tea and its flavonoids to improve endothelial function and lower blood pressure provide a likely mechanism for benefits on cardiovascular health.[8] A higher flavonoid intake and intake of green and black teas are associated with a 10−20% lower risk of coronary heart disease (CHD) and stroke.[9] Tools for obesity management including consumption of caffeine and different teas have been proposed as strategies for weight loss and weight maintenance, because they may increase energy expenditure (4−5%) and fat oxidation (10−16%) and have been proposed to counteract the decrease in metabolic rate that is present during weight loss.[10]

Black tea is processed in either of two ways: orthodox or CTC (crush, tear, and curl).[11,12] It is usually graded on one of

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four scales of quality (such as whole leaf, broken leaf, fannings, and dust). In Turkey, >50% of black tea is processed by the ÇAYKUR Tea Processing Plant, which processes black tea according to its own seven different grades [high-quality tea (grades 1−3) and low-quality tea (grades 4−7)].[47] The low-quality groups of teas are marketed after being blended with high-quality group categories according to the demands. It is, therefore, of great interest to observe the differences among different grades of black tea in terms of their nutritional composition and functional characteristics. The objective of this study was to compare the compositional, nutritional, and functional characteristics of seven grades of black tea.

MATERIALS AND METHODS

Samples. Seven grades of black tea according to their processing were procured from the ÇAYKUR Tea Processing Plant in Rize at the beginning of the first harvest season of June 2011. Graded teas (10 kg from each grade) were obtained from the same processing line to make a true comparison. They were kept in their pack in a temperature-controlled cabinet (at ∼5 °C with a relative humidity of 65−70%) at the Food Institute (TÜBİTAK Marmara Research Center, Gebze, Turkey) until they were analyzed. All samples were analyzed within 3 months of arrival.

Reagents and Standards. All chemical reagents were obtained from Sigma-Aldrich-Fluka Co. Ltd. (Prolab, Istanbul, Turkey), unless otherwise stated.

Determination of Proximate Composition. Percentages of moisture by vacuum oven (method 934.06), total fat by Soxhlet extraction (method 920.39C), protein by Kjeldahl nitrogen (method 920.152), and ash by direct analysis (method 940.26) were determined according to AOAC methods.[13] Percentage crude protein was estimated by multiplying the total nitrogen content by a factor of 6.25.[13] Total carbohydrates were calculated by subtracting the total percentage of other components from 100.

Determination of Dietary Fiber. Total fiber, soluble fiber, and insoluble fiber were determined by using the AOAC enzymatic−gravimetric method (991.43).[13] The oven-dried tea (at 105 °C for 24 h) was defatted three times each with petroleum ether (10 mL/g). The samples were then dried overnight at 40 °C. Finally, the flow diagram outlined by AOAC procedure was followed. Crude protein was calculated as total nitrogen × 6.25.

Determination of Minerals. Minerals were analyzed according to AOAC method 999.10.[13] Tea sample (1 g) was weighed into Teflon microwave digestion vessel, and 5 mL of concentrated HNO_3 and 1 mL of H_2O_2 were added. The vessel was then closed and placed into a microwave oven. Subsequently, clear digested sample after cooling was transferred to a 50 mL volumetric flask and made up to a final volume of 50 mL with the deionized water. Minerals were determined using an inductively coupled plasma-mass spectrometer (ICP-MS) (Elan DRC-E, Perkin-Elmer, Norwalk, CT, USA). The following isotopes were used to quantify the minerals: calcium, 44; chromium, 53; copper, 63; cobalt, 59; iron, 57; magnesium, 26; manganese, 55; molybdenum, 98; phosphorus, 31; potassium, 39; selenium, 78; sodium, 23; and zinc, 66.

Determination of Water-Soluble Vitamins. Briefly, 1 g of grated tea sample was extracted with 10 mL of hot water (80 °C) for 10 min. The supernatant was collected into a flask after centrifugation at 7500*g* for 5 min. The residue was further extracted with 5 mL of hot water (80 °C) two times. The combined extract was diluted with water at a ratio of 1:5 (v/v), and 1 mL of extract was passed through a 0.45 μm nylon filter and transferred into a high-performance liquid chromatography (HPLC) vial. The water-soluble vitamins in tea were analyzed with ultraperformance liquid chromatography−tandem mass spectrometer (UPLC-MS/MS) (Waters Corp., Milford, MA, USA) as described by a Waters Corp. Application Note.[14] The black tea extracts were separated by using a reversed phase Acquity UPLC (flow rate of 0.3 mL/min) on a BEH C_{18} column (Waters Corp., Dublin, Ireland), having a 1.7 μm particle size (i.d. = 2.1 mm, length = 100 mm) at 40 °C. The injection volume was set to 20 μL. The binary mobile phase consisted of solvent A, composed of formic acid 0.1% in HPLC grade water, and solvent B, composed of formic acid 0.1% in methanol. The solvent gradient was programmed as follows: isocratic elution of 1% B and 99% A, 0−3 min; linear gradient elution to 75% B and 25% A, 3−5 min; linear gradient elution to 1% B and 99% A, 5−5.1 min; and isocratic elution of 1% B and 99% A, 5.1−8 min.

In MS/MS detections, the positive ionization mode (negative for only ascorbic acid) was used, and the ions were monitored and performed with a capillary voltage of 1 kV. The source block and desolvation temperatures were set at 120 and 450 °C, respectively, whereas the desolvation and cone gas (N_2) flow rates were set at 800 and 10 L/h, respectively. Cone voltage and collision energy were optimized for each compound separately. MS/MS ionization conditions of the water-soluble vitamins analyzed are given in Table 1. MS/MS data were collected and processed with MassLynx software version 4.1 from Waters Inc. (Waters Corp.). MS/MS data acquisition from each tea sample was carried out using the multiple reaction monitoring (MRM) mode. The areas from the peaks corresponding to parent ions were used for the quantification of single vitamins. All analyses were performed in triplicate, and the reported quantitative data presented here are the averages of three measurements. Values were expressed as milligrams per 100 g of tea.

Table 1. MS/MS Conditions for Water-Soluble Vitamins

vitamin	ionization mode	parent ion (*m*/*z*)	daugther ion (*m*/*z*)	cone voltage (V)	collision energy (eV)
thiamin	ESI+	265.1	122	22	14
niacin	ESI+	123.15	80	36	17
pyridoxine	ESI+	170.1	152.05	26	12
pantothenic acid	ESI+	220.15	90	23	15
ascorbic acid	ESI-	175.1	115	25	13
cyanocobalamin	ESI+	678.45	147.1	37	36

Determination of Total Phenolic Content. The content of total phenolics was determined according to the procedure described by ISO 14502-2:2005, using the Folin−Ciocalteu phenol reagent.[15] Phenolics were extracted with 70% methanol, and absorbance was read using a microplate reader (FLUOStar Omega, BMG Labtech, Ortenberg, Germany). The content of total phenolics was calculated from a standard curve using gallic acid as a standard and expressed as grams of gallic acid equivalents (GAE) per 100 g of tea.

Determination of Oxygen Radical Absorbance Capacity (ORAC). The antioxidant activity was determined according to ORAC assay as described by Wu et al.[16] Samples were extracted by acetone/water/acetic acid (70:29.5:0.5, v/v/v), and the analysis was performed using a microplate reader (FLUOStar Omega, BMG Labtech). ORAC values were calculated by using the trolox and sample concentration and the net area under the fluorescein decay curve (AUC). Data were expressed as micromoles of trolox equivalents (TE) per gram of tea.

Determination of Trolox Equivalent Antioxidant Capacity (TEAC). The antioxidant activity in tea extract was measured according to the TEAC assay as described by Dubeau et al.[17] Samples were extracted by 70% methanol as mentioned by ISO 14502-2:2005,[15] and the analysis was performed on the diluted samples using a microplate reader (FLUOStar Omega, BMG Labtech). TEAC values were calculated by using trolox as standard, and data were expressed as micromoles of TE per gram of tea.

Determination of Cupric Ion Reducing Antioxidant Capacity (CUPRAC). The method described by Apak et al.[18] was used to assess the CUPRAC extract and its fraction. Samples were extracted by 70% methanol as mentioned by ISO 14502-2:2005,[15] and the analysis was performed on the diluted samples as described by Apak et al.,[18] using a microplate reader (FLUOStar Omega, BMG Labtech). CUPRAC values were calculated by using the trolox as standard, and data were expressed as micromoles of TE per gram of tea.

Determination of Phenolic Compounds. Phenolic compounds were extracted and analyzed according to the method described by Dou et al.,[19] with some modifications. One gram of graded tea sample

was extracted with 10 mL of hot water (80 °C) for 10 min. The supernatant was collected into a flask after centrifugation at 7500*g* for 5 min. The residue was further extracted with 5 mL of hot water (80 °C) two times. The combined extract was diluted with water at a ratio of 1:5 (v/v), and 1 mL of extract was passed from a 0.45 μm nylon filter and transferred into a HPLC vial. Chromatographic analyses were performed on an Agilent 1200 HPLC system consisting of a photodiode array detector (DAD), quaternary pump, autosampler, and column oven (Agilent Technologies, Waldbronn, Germany). An Agilent Zorbax Bonus-RP-C18 column (150 mm × 4.6 mm, 3.5 μm) was used to separate flavanols, alkoloids, and phenolic acids in tea extract. A linear gradient elution program with a mobile phase containing solvent A (acetonitrile) and solvent B (acetic acid/H_2O, 0.1:99.9, v/v) was used at a flow rate of 1 mL/min. The solvent gradient was programmed as follows: linear gradient elution from 10 to 20% A (0−15 min), then linear gradient elution from 20 to 40% A (15−25 min), and linear gradient elution from 40 to 10% A (25−30 min). Ten microliters of the sample was injected automatically at 25 °C. Chromatograms were recorded at 280 nm with spectra (200−700 nm) taken continuously throughout the elution. Identifications of flavanols, alkoloids, and phenolic acids were accomplished by comparing the retention time and absorption spectra of peaks in tea sample to those of standard compounds. The quantitation of flavanols, alkoloids, and phenolic acids were based on calibration curves built for each of the compounds identified in tea grades. Data were expressed as milligrams per 100 g of tea.

Determination of Thearubigins. *Preparation of Thearubigin Standard.* Thearubigins were extracted according to the caffeine precipitation method of Kuhnert et al.[20] Briefly, 8 g of graded tea sample was extracted with 150 mL of hot water (80 °C) and kept in a water bath for 10 min. The sample extract was then filtered through a Whatman no. 4 filter paper. After that, caffeine sufficient to achieve 20 mM was added to the supernatant and stirred, and the mixture was allowed to stand at 4 °C for 2 h. It was then centrifuged at 4000*g* (Sigma 2-16PK, Osterode am Harz, Germany) for 40 min at room temperature. The resulting precipitate was suspended in hot water and washed five times with 40 mL of ethyl acetate until no color remained in the extract. Then, ethyl acetate supernatant was removed and evaporated to dryness at room temperature under nitrogen, and the remaining theaflavin residue was recovered with 10 mL of distilled water. Finally, the aqueous phase was partitioned at 80 °C with 2 volumes of chloroform, and the decaffeinated liquid was stored at −80 °C and then freeze-dried. The freeze-dried thearubigins were obtained as orange to light brown colored fluffy powder and used as reference standard material in this study.

Sample Extraction. Thearubigins in tea samples were extracted according to procedure described by Tanaka et al.[21] Two grams of graded tea sample was extracted twice with 50 mL of 70% acetone in an ultrasonic bath at room temperature. Acetone was removed with a rotary evaporator (Büchi Labortechnik AG, Flawil, Switzerland), and the volume of resulting aqueous solution was adjusted to 50 mL. The solution was partitioned with 50 mL of ethyl acetate three times, and then the ethyl acetate solution was combined and concentrated to dryness. The residue was dissolved in 10 mL of ethanol before analysis.

The aqueous phase remaining after solvent partitioning was concentrated with a rotary evaporator (Büchi Labortechnik AG) to remove residual ethyl acetate. The resulting solution was acidified by the addition of a few drops of trifluoroacetic acid (pH <4), and the volume was adjusted to 100 mL. A 20 mL portion was passed through a Sep-Pak ODS cartridge (Waters Corp.), and polyphenols adsorbed on the cartridge were eluted with 80% of ethanol. The volume of elute was adjusted to 10 mL and analyzed by ultrafast liquid chromatography (UFLC). Thearubigins were quantified on the basis of peak area and comparison with a calibration curve obtained with the corresponding reference standard material obtained by caffeine precipitation method. [20]

Both the sample extracts and the reference standard were analyzed according to the same procedure. Twenty microliters of the sample was automatically injected into a Kromosil C-18 column (150 × 4.6 mm, 5 μm particles, Teknokroma, Barcelona, Spain) at 35 °C. The equipment consisted of a Shimadzu LC-20AD pump, an SPD-M20A DAD, an SIL-20A HT autosampler, a CTO-2OAC column oven, a DGU-20A_5 degasser, and a CMB-20A communications bus module (Shimadzu Corp., Kyoto, Japan). A gradient of mobile phases A (acetonitrile) and B (50 mM phosphoric acid) at a flow rate of 0.8 mL/min was used for the chromatographic separation. The gradient profile was programmed as follows: 0−29 min, linear gradient from 10 to 23% A; 29−30 min, linear gradient from 23 to 90% A; and 30−40 min, isocratic elution 90% A. Data were expressed as milligrams of thearubigins per 100 g of tea.

Determination of Theaflavin. Theaflavin was extracted according to the methods of Neilson et al.[22] and Mulder et al.,[23] with some modifications. In brief, 5 g of graded tea sample was brewed in 250 mL of hot water (80 °C) for 5 min (by mild stirring). After this process, 20 mL of each infusion was diluted with 10 mL of acetic acid solution to lower the pH and stabilize the polyphenol components prior to analysis. Acidified solutions were centrifuged for 10 min at 4000*g* value (Sigma 2-16PK). Aliquots of each supernatant were then collected and stored at −80 °C until analysis was carried out. Theaflavin was quantified on the basis of peak area and comparison with a calibration curve obtained with the corresponding standard.

The equipment consisted of a UFLC (Prominence Liquid Chromatograph LC-20AD, Shimadzu Corp.) coupled with tandem mass spectrometry (MS/MS) (API-2000 Liquid Chromatography/Tandem Mass Spectrometry System, ABSciex, Framingham, MA, USA). Twenty microliters of the sample was automatically injected into a Luna Phenyl Hexyl (250 × 4.6 mm, 5 μm particles, Phenomenex, Cheshire, UK) at 30 °C. A gradient of mobile phases A (water/acetonitrile/acetic acid, 96:2:2, v/v) and B (acetonitrile/acetic acid, 98:2, v/v) was used, at a flow rate of 0.5 mL/min, for the chromatographic separation. The gradient profile was programmed as follows: 0−10 min, 95% B isocratic eluent; 11−13 min, isocratic elution 15% B. Data were expressed as milligrams of theaflavin per 100 g of tea.

Determination of Chlorophylls and Carotenoids. Chlorophylls, carotenoids, and their derivatives were extracted and analyzed according to the method described by Gökmen et al.,[24] with some minor modifications. Briefly, 1 g of grated tea sample was mixed with 100 mg of sodium carbonate and extracted with 10 mL of methanol for 10 min. The organic phase was pooled into a flask after centrifugation at 7500*g* for 5 min. The residue was further extracted with 10 mL of methanol four times until a colorless extract was achieved. One milliliter of the combined organic phases passed from a 0.45 μm nylon filter and transferred into a HPLC vial. Chromatographic analyses were performed on an Agilent 1200 HPLC system consisting of a DAD, quaternary pump, autosampler, and column oven (Agilent Technologies). An Agilent Eclipse XDB-C8 column (150 mm × 4.6 mm, 5 μm) was used to separate chlorophylls, carotenoids, and their derivatives. An isocratic elution was carried out at 25 °C with a mobile phase containing 95% methanol and 5% HPLC grade water at a flow rate of 0.75 mL/min. Ten microliters of the sample was injected, and the separation of chlorophylls, carotenoids, and their derivations was completed in 35 min. Chromatograms were recorded simultaneously at 450 nm (lutein and *β*-carotene), 652 nm (chlorophyll *b*), and 666 nm (chlorophyll *a*), with spectra (400−700 nm) taken continuously throughout the elution. Identifications of *β*-carotene, lutein, and chlorophylls *a* and *b* were accomplished by comparing the retention time and absorption spectra of peaks in tea samples to those of standard compounds. The quantitations of *β*-carotene, lutein, and chlorophylls *a* and *b* were based on calibration curves built for each of the compounds identified in tea samples. Data were expressed as milligrams per 100 g of tea.

Statistical Analysis. Results were expressed as the mean ± standard deviation (SD) (n = 3) for each analysis. Differences were estimated by analysis of variance (ANOVA) followed by Tukey's "Honest Significant Difference" test. Differences were considered to be significant at $p \leq 0.05$. All statistical analyses were performed using the SPSS 18.0 version (SPSS Inc., Chicago, IL, USA).

Table 2. Proximate Composition and Dietary Fiber of Seven Grades of Black Tea (Grams per 100 g)[a]

	grade 1	grade 2	grade 3	grade 4	grade 5	grade 6	grade 7
moisture	4.58 ± 0.01e	5.24 ± 0.00b	5.30 ± 0.03a	4.58 ± 0.01e	5.02 ± 0.01c	5.04 ± 0.03c	4.84 ± 0.01d
ash	4.76 ± 0.00b	4.70 ± 0.01c	4.61 ± 0.01d	4.61 ± 0.03d	4.55 ± 0.01e	4.45 ± 0.03f	5.55 ± 0.01a
protein	21.63 ± 0.09d	22.81 ± 0.00a	22.00 ± 0.08b	21.81 ± 0.00c	22.72 ± 0.04a	21.22 ± 0.04e	21.57 ± 0.09d
dietary fiber	49.68 ± 0.05d	50.06 ± 0.20d	54.19 ± 0.03a	51.98 ± 0.12c	52.93 ± 0.26b	54.31 ± 0.32a	52.67 ± 0.27b
soluble fiber	4.78 ± 0.11e	5.72 ± 0.08bc	5.21 ± 0.14d	5.25 ± 0.10d	5.42 ± 0.08cd	5.90 ± 0.01ab	6.08 ± 0.25a
insoluble fiber	44.90 ± 0.16f	44.34 ± 0.28e	48.98 ± 0.17a	46.73 ± 0.22d	47.51 ± 0.18c	48.41 ± 0.33b	46.59 ± 0.01d
fat	0.28 ± 0.01ab	0.16 ± 0.01c	0.16 ± 0.01c	0.12 ± 0.01d	0.09 ± 0.02d	0.30 ± 0.02a	0.26 ± 0.01b
carbohydrate	19.09 ± 0.16a	17.03 ± 0.17b	13.74 ± 0.08d	16.91 ± 0.13b	14.70 ± 0.25c	14.69 ± 0.30c	15.13 ± 0.37c

[a]Data are expressed as the mean ± SD (n = 3) on a fresh weight basis. Means ± SD follow by the same letter, within a row, are not significantly different (p > 0.05).

Table 3. Mineral and Vitamin Contents of Seven Grades of Black Tea (Milligrams per 100 g)[a]

	grade 1	grade 2	grade 3	grade 4	grade 5	grade 6	grade 7
mineral							
calcium	454 ± 52a	481 ± 44a	466 ± 13a	451 ± 41a	520 ± 74a	483 ± 11a	543 ± 82a
chromium	0.19 ± 0.02b	0.16 ± 0.02b	0.17 ± 0.00b	0.17 ± 0.02b	0.14 ± 0.02bc	0.11 ± 0.01c	0.26 ± 0.05a
copper	0.91 ± 0.11bc	1.03 ± 0.11ab	0.91 ± 0.05bc	0.86 ± 0.07bc	0.96 ± 0.11bc	0.84 ± 0.02c	1.19 ± 0.15a
cobalt	0.04 ± 0.01b	0.05 ± 0.00b	0.04 ± 0.01bc	0.03 ± 0.00bc	0.04 ± 0.00bc	0.03 ± 0.00c	0.07 ± 0.01a
magnesium	155 ± 20b	170 ± 16b	158 ± 5b	163 ± 15b	178 ± 25b	155 ± 5b	222 ± 32a
manganese	115 ± 8a	123 ± 8a	106 ± 61a	116 ± 12a	124 ± 49a	114 ± 7a	112 ± 10a
molybdenum	0.10 ± 0.03a	0.04 ± 0.00b	0.02 ± 0.00c	0.02 ± 0.00c	0.01 ± 0.00c	0.01 ± 0.00c	0.01 ± 0.00c
iron	31.41 ± 1.10b	24.26 ± 1.14c	24.34 ± 5.16c	23.75 ± 0.31c	18.79 ± 1.70d	16.75 ± 0.52d	48.74 ± 3.50a
phosphorus	257 ± 19d	302 ± 6a	274 ± 9cd	279 ± 2bc	314 ± 10a	298 ± 10ab	303 ± 15a
potassium	1707 ± 123a	1769 ± 110a	1774 ± 121a	1812 ± 15a	1699 ± 169a	1534 ± 43a	1753 ± 226a
selenium	0.07 ± 0.01c	0.08 ± 0.01bc	0.09 ± 0.01b	0.09 ± 0.01b	0.09 ± 0.00bc	0.09 ± 0.01b	0.11 ± 0.01a
sodium	1.94 ± 0.09b	2.00 ± 0. 21b	1.97 ± 0.23b	1.98 ± 0.37b	1.72 ± 0.16b	1.60 ± 0.15b	2.95 ± 0.42a
zinc	1.65 ± 0.12cd	1.90 ± 0.15b	1.70 ± 0.08cd	1.54 ± 0.10cd	1.74 ± 0.14bc	1.51 ± 0.02d	2.20 ± 0.11a
vitamin							
thiamin	0.01 ± 0.00b	0.01 ± 0.00b	0.02 ± 0.00a	0.02 ± 0.00a	0.01 ± 0.00b	0.01 ± 0.00b	0.02 ± 0.00a
niacin	1.98 ± 0.02b	1.94 ± 0.02b	2.14 ± 0.03ab	1.96 ± 0.14b	1.98 ± 0.02b	1.94 ± 0.00b	2.24 ± 0.05a
pantothenic acid	9.38 ± 0.15ab	7.60 ± 0.73ef	8.93 ± 0.01b	8.30 ± 0.01d	8.60 ± 0.00c	8.07 ± 0.03e	9.53 ± 0.01a
pyridoxine	0.25 ± 0.00a	0.14 ± 0.07b	0.25 ± 0.01a	0.26 ± 0.01a	0.24 ± 0.02a	0.26 ± 0.03a	0.27 ± 0.01a

[a]Data are expressed as the mean ± SD (n = 3) on a fresh weight basis. Means ± SD follow by the same letter, within a row, are not significantly different (p > 0.05).

RESULTS AND DISCUSSION

Proximate Composition and Dietary Fiber. Table 2 shows the proximate composition and dietary fiber content in seven grades of black tea. Insoluble dietary fiber was the predominant component (ranging from 44.34 to 48.98 g/100 g), followed by protein (ranging from 21.22 to 22.81 g/100 g), and carbohydrate (ranging from 13.74 to 19.09 g/100 g). Moisture, ash, and, to a lesser extent, fat were also present in all grades of black tea. Some variations among seven grades of black tea were observed (p < 0.05). The values reported herewith were comparable with those reported in the literature for black tea.[25]

Minerals. Thirteen minerals (calcium, chromium, copper, cobalt, iron, magnesium, manganese, molybdenum, phosphorus, potassium, selenium, sodium, and zinc) were studied for the first time in seven grades of black tea, and the results are given in Table 3. Among the minerals, potassium was most abundant followed by calcium, phosphorus, and magnesium. No significant differences (p > 0.05) in calcium, manganese, and potassium contents among the seven tea grades were observed. Some variations (p < 0.05) for other minerals were noted. Mineral composition of black tea may be affected by variety, geographical origin, harvest time and year, climate, composition of soil, use of fertilizer, method of cultivation, and processing of tea, among others. The mineral content in seven grades of black tea is within the range of Food Composition and Nutrition Tables.[25]

Water-Soluble Vitamins. Four water-soluble vitamins (thiamin, niacin, pantothenic acid, and pyridoxine) were detected in seven grades of black tea (Table 3). Pantothenic acid was most abundant (ranging from 7.60 to 9.53 mg/100 g). Significant differences in each water-soluble vitamin consitutents (p < 0.05) existed among the seven grades of black tea, with some exceptions. No systematic classification could be attributed to vitamin contents among high- and low-quality black tea grades. Vitamin C and cyanocobalamin were not detected in any of the black tea grades. Souci et al.[25] reported five water-soluble vitamins in black tea (thiamin, riboflavin, niacin, pantothenic acid, and pyridoxine), of which niacin was most abundant (7.7 mg/100 g), followed by pantothenic acid (1.3 mg/100 g), riboflavin (0.950 mg/100 g), pyridoxine (0.250 mg/100 g), and thiamin (0.025 mg/100 g). Riboflavin was also not detected in any grades of black tea. The observed differences could be due to varietal or processing.

Total Phenolics and Antioxidant Activities. Total phenolic content and antioxidant activities using three different assays (ORAC, TEAC, and CUPRAC) were measured in seven grades of black tea (Table 4). Total phenol contents ranged

Table 4. Total Phenolic Content (Grams of GAE per 100 g) and Antioxidant Activities (Micromoles of TE per Gram) of Seven Grades of Black Tea[a]

	grade 1	grade 2	grade 3	grade 4	grade 5	grade 6	grade 7
total phenolics	8.29 ± 0.15a	8.18 ± 0.20a	8.14 ± 0.55a	7.75 ± 0.12ab	7.79 ± 0.37ab	7.52 ± 0.12b	8.23 ± 0.35a
ORAC	1173 ± 57ab	1075 ± 136abc	1210 ± 98a	983 ± 33c	1051 ± 152bc	795 ± 56d	777 ± 106d
TEAC	809 ± 5a	726 ± 23bc	722 ± 25bc	726 ± 30bc	757 ± 33ab	688 ± 47c	769 ± 49 ab
CUPRAC	755 ± 30a	709 ± 39ab	669 ± 40bcd	686 ± 12bc	646 ± 28cd	615 ± 35d	721 ± 33ab

[a]Data are expressed as the mean ± SD (n = 3) on a fresh weight basis. Means ± SD follow by the same letter, within a row, are not significantly different (p > 0.05). Abbreviations: ORAC, oxygen radical absorbance apacity; TEAC, trolox equivalents antioxidant capacity; CUPRAC, cupric ion reducing antioxidant capacity.

from 7.52 to 8.29 g of GAE/100 g, being lowest in grade 6 and highest in grade 1. With respect to antioxidant activities, a large variation in ORAC values was observed among all grades of black tea (ranging from 777 μmol of TE/g in grade 7 to 1210 μmol of TE/g in grade 3). The ORAC values for high-quality black tea (grades 1−3) showed significantly higher (p < 0.05) values compared to the low-quality black teas (grades 4−7), with some exceptions. The values for TEAC (varying from 688 to 809 μmol of TE/g) and CUPRAC (varying from 615 to 755 μmol of TE/g) were not significantly different (p > 0.05) between low- and high-quality tea, with some exceptions.

Cao et al.[26] measured the antioxidant capacity of black and green teas as well as 22 common vegetables and found that black tea had a much higher ORAC value (927 μmol of TE/g on a dry weight basis) against peroxyl radicals than green tea and any of the vegetable studied. Their finding is within the range of ORAC values found in the seven grades of black tea (777−1210 μmol of TE/g on a fresh weight basis). The average moisture content of black tea was around 5% (Table 2).

Phenolics. A typical phenolic profile identified by HPLC in the seven grades of black tea is shown in Figure 1. The contents

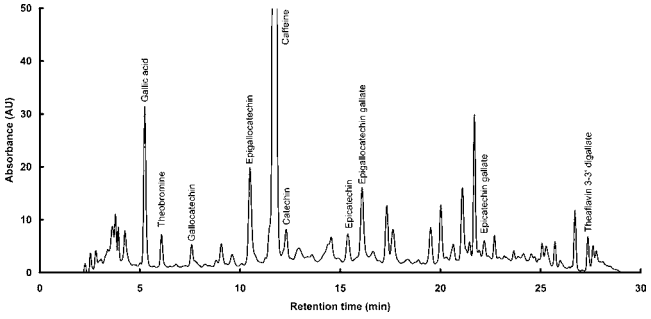

Figure 1. Typical phenolic profiles indentified by HPLC in seven grades of black tea.

of phenolics, including six major flavanols (catechin, epicatechin, epicatechin gallate, epigallocatechin, epigallocatechin gallate, and gallocatechin), two alkaloids (caffeine and theobromine), three condensed phenolics (theaflavin, thearubigins, theaflavin 3,3′-digallate), and one phenolic acid (gallic acid) in the seven grades of black tea are given in Table 5. Some variations (p < 0.05) in phenolics were noted among the seven grades of black tea, with some exceptions. Despite the fact that there was no clear trend, high-quality black teas (grades 1−3) had a tendency to have higher flavanols, alkoloids, and condensed phenolics than low-quality black teas (grades 4−7). The variations of phenolic constituents could be attributed to the varying leaf quality and the different plucking intervals in each shooting period.[27]

Flavanols. Among flavanols, epigallocatechin was predominant (ranging from 1038 to 1214 mg/100 g), followed by gallocatechin (ranging from 467 to 650 mg/100 g), epigallocatechin gallate (ranging from 102 to 155 mg/100 g), and, to lesser extents, catechin, epicatechin, and epicatechin gallate. Flavanols detected in the present study were also reported in various black teas, albeit to different extents.[28−32]

Epigallocatechin contents in seven grades of black tea were higher than those reported in the literature.[32−34] In contrast, epicatechin gallate levels were lower than those reported in the literature.[32,35,36] In addition, epigallocatechin gallate have been reported between 270 and 552 mg/100 g in various types of black teas,[32,37,38] which were higher than in the present study (Table 5). Despite the fact that gallocatechin was the second most abundant flavanol in the seven grades of black tea, its level was reported to be lower (70 mg/100 g dry weight) than present values for black teas.[29]

Alkaloids. Beside flavanoids, alkaloids are also important biologically active constituents of black tea. Caffeine and theabromine were present, whereas theophylline was not detected in any grades of black tea (Table 5). Caffeine was the dominant alkaloid, ranging from 1525 to 1806 mg/100 g. No significant differences (p > 0.05) were found in theabromine (ranging from 12.6 to 15.4 mg/100 g) among the seven grades of black tea.

Sharma et al.[37] reported the content of theabromine in orthodox tea as 106 mg/100 g, which was higher than the present results, whereas caffeine content was 1790 mg/100 g, which was in agreement with the levels found in this study (Table 5). The caffeine levels found in the seven grades of black tea (ranging from 1525 to 1806 mg/100 g) were lower than those published in the literature for different types of black teas (ranging from 2100 to 3900 mg/100 g).[27,39,40] Several factors including fermentation, extraction solvent, and drying stages may affect this.[40] Presently, the major biosynthetic route of caffeine is considered to be a xanthosine−7-methylxanthosine−7-methylxanthine−theobromine−caffeine pathway.[41] In addition, Deng et al.[42] concluded that caffeine biosynthesis was mainly controlled by the first *N*-methyl-transfer reaction catalyzed by 7-methylxanthosine synthase. Hence, the relatively lower caffeine and theabromine contents for seven grades of black tea might be attributed either to the lower supply of xanthosine for caffeine biosynthesis or to the lower activity of the 7-methylxanthosine synthase.

Condensed Phenolics. Figures 2 and 3 show typical chromatograms of theaflavin and thearubigins identified in the seven grades of black tea, respectively. Among the condensed phenolics, thearubigins were dominant (ranging from 5920 to 6830 mg/100 g) followed by theaflavin (ranging from 121 to 399 mg/100 g) and, to a lesser extent, theaflavin 3,3′-digallate (ranging from 12.5 to 18.1 mg/100 g). Some

Table 5. Phenolics (Milligrams per 100 g) in Seven Grades of Black Tea[a]

	grade 1	grade 2	grade 3	grade 4	grade 5	grade 6	grade 7
flavanols							
catechin	89.6 ± 5.6ab	98.3 ± 1.8a	86.1 ± 2.7b	90.3 ± 1.6ab	93.9 ± 2.2ab	75.1 ± 3.4c	59.3 ± 4.0d
epicatechin	83.9 ± 7.0a	78.8 ± 6.2ab	70.2 ± 5.9bc	66.5 ± 1.8bc	69.6 ± 2.9bc	61.9 ± 1.1c	62.6 ± 0.8c
epicatechin gallate	103 ± 7ab	115 ± 6a	100 ± 5ab	93.7 ± 4.9b	102 ± 6.3ab	89.5 ± 6.3b	99.3 ± 1.6ab
epigallocatechin	1214 ± 18a	1141 ± 52ab	1065 ± 34b	1091 ± 59ab	1042 ± 68b	1038 ± 55b	1127 ± 29ab
epigallocatechin gallate	111 ± 2d	155 ± 3a	122 ± 2c	105 ± 4de	131 ± 3b	102 ± 2e	108 ± 3de
gallocatechin	650 ± 38a	624 ± 7a	524 ± 13b	506 ± 13bc	524 ± 26b	467 ± 11c	530 ± 11b
alkoloids							
caffeine	1695 ± 21b	1806 ± 5a	1626 ± 6bc	1596 ± 37cd	1624 ± 41bc	1525 ± 28d	1689 ± 27b
theabromine	14.6 ± 2.1a	15.3 ± 0.4a	15.4 ± 2.5a	14.7 ± 0.1a	14.2 ± 0.9a	13.4 ± 0.5a	12.6 ± 1.9a
condensed phenolics							
theaflavin	298 ± 18b	399 ± 27a	288 ± 47b	243 ± 22b	184 ± 16c	121 ± 3d	180 ± 9c
theaflavin 3,3′-digallate	15.9 ± 0.4a	18.1 ± 1.8a	17.3 ± 1.8a	15.6 ± 0.4a	12.5 ± 1.9a	15.4 ± 0.1a	15.0 ± 3.2a
thearubigins	6830 ± 260a	6790 ± 130a	6360 ± 200a	5950 ± 170a	5940 ± 280a	5920 ± 170a	6006 ± 90a
phenolic acids							
gallic acid	96.8 ± 9.3a	110 ± 11a	100 ± 9a	99.0 ± 6.9a	105 ± 10a	100 ± 8a	116 ± 10a

[a]Data are expressed as the mean ± SD (n = 3) on a fresh weight basis. Means ± SD follow by the same letter, within a row, are not significantly different (p > 0.05).

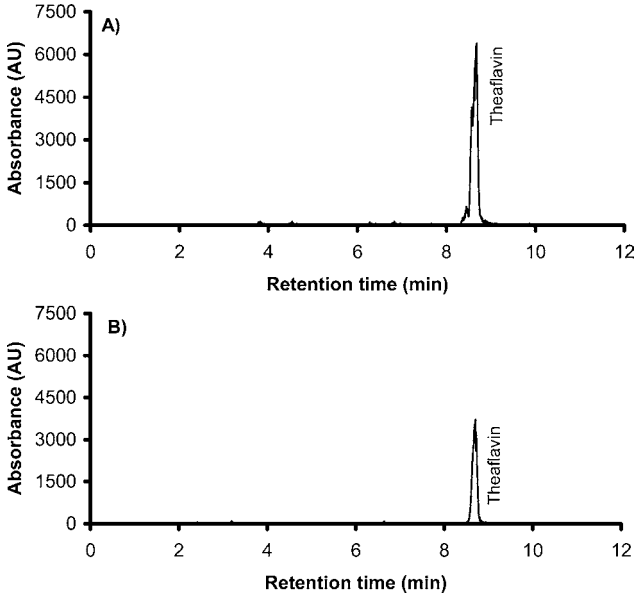

Figure 2. Typical chromatogram of theaflavin in seven grades of black tea (A) and theaflavin standard (B).

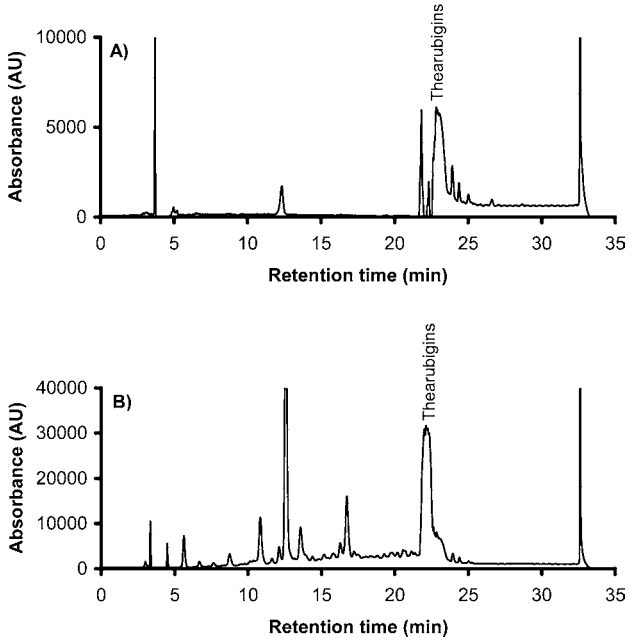

Figure 3. Typical chromatogram of thearubigins in seven grades of black tea (A) and reference standard of thearubigins (B).

variations (p < 0.05) in theaflavin were noted, whereas no significant differences (p > 0.05) existed in theaflavin 3,3′-digallate and thearubigins among the seven grades of black tea.

Black tea contains different extents and variety of condensed phenolic compounds owing to the polymerization reactions of monomeric phenolic substances.[43] Liang et al.[36] reported that the total amount of theaflavin in 17 black tea samples ranged from 117 to 2510 mg/100 g, with an average of 688 g/100 g. Theaflavin levels have been reported to vary between 40 and 910 mg/100 g for black tea samples extracted with different solvents.[22,28,44] These values are in accordance with the current data (ranging from 121 to 399 mg/100 g) for the seven grades of black tea. The thearubigins, which are the polymeric form of oxidized flavanols, are the dominant phenolics of black tea.[3] Balentine[28] reported that black tea leaves contained 5950 mg/100 g thearubigins (dry weight), which was within the range of the present study (Table 5). Recently, Tanaka et al.[21] found that thearubigin levels in a new fermented tea were 2031 mg/100 g (dry leaves).

Phenolic Acids. Gallic acid was the only phenolic acid detected in the seven grades of black tea, being lowest in grade 1 (96.8 mg/100 g) and highest in grade 7 (116 mg/100 g). No significant differences (p > 0.05) existed among the seven grades of black tea (Table 5). The content of gallic acid in the present study was lower than those of Darjeeling (262 mg/100 g) and Yingde (169 mg/100 g) black teas.[45] The concentration and composition of phenolic acids in tea products depend not only on differences in processing but also on tea plant cultivars and agricultural conditions.[3]

Chlorophylls and Carotenoids. Table 6 shows the levels of chlorophylls and carotenoids in the seven grades of black tea. Chlorophyll *a*, chlorophyll *b*, and total chlorophyll ranged from 125.7 to 228.2 mg/100 g, from 33.1 to 54.0 mg/100 g, and

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Table 6. Chlorophylls and Carotenoids (Milligrams per 100 g) in Seven Grades of Black Tea[a]

	grade 1	grade 2	grade 3	grade 4	grade 5	grade 6	grade 7
chlorophyll *a*	213.3 ± 4.3d	228.2 ± 6.9e	200.1 ± 3.9c	209.7 ± 2.8d	185.3 ± 0.3b	178.3 ± 5.2b	125.7 ± 4.4a
chlorophyll *b*	48.5 ± 0.5b	54.0 ± 1.3c	50.8 ± 1.8bc	50.4 ± 1.9bc	47.8 ± 1.7b	48.5 ± 1.3b	33.1 ± 3.0a
total chlorophyll	261.8 ± 4.8d	282.3 ± 8.2e	250.9 ± 5.7c	260.1 ± 4.7cd	233.1 ± 2.1b	226.8 ± 6.5b	158.8 ± 7.4a
lutein	24.1 ± 0.5b	25.8 ± 0.9c	25.1 ± 0.6bc	24.3 ± 0.5b	23.9 ± 0.2b	24.2 ± 0.3b	16.4 ± 0.4a
β-carotene	7.4 ± 0.3b	8.3 ± 0.3c	8.2 ± 0.2c	7.6 ± 0.1b	7.6 ± 0.1b	7.9 ± 0.2bc	3.9 ± 0.1a

[a]Data are expressed as the mean ± SD (n = 3) on a fresh weight basis. Means ± SD follow by the same letter, within a row, are not significantly different (p > 0.05).

from 158.8 to 282.3 mg/100 g, respectively. The contents of chlorophyll *a* were found to be 4−5-fold higher than that of corresponding chlorophyll *b* counterparts. Lutein (16.4−25.8 mg/100 g) was a more abundant carotenoid than *β*-carotene (3.9−8.3 mg/100 g) in all grades of black tea. In general, high-quality black tea (grades 1−3) contained significantly higher (p < 0.05) chlorophylls and carotenoids as compared to the low-quality black teas (grades 4−7). Ravichandran[46] reported higher *β*-carotene (16.6 mg/100 g) and lower lutein (16.8 mg/100 g) in orthodox black tea than reported values herein. They also reported that total chlorophyll content of green tea leaves was 1471 mg/100 g, which declined to 129 mg/100 g in orthodox black tea at the end of the processing. The levels of carotenoids and chlorophylls in black tea depend not only on differences in processing but also on tea plant cultivars and agricultural conditions.

The present work suggests that high- and low-quality black teas should not be distinguished on the basis of their nutritional and functional characteristics. Despite the fact that low-quality black tea had a tendency to have lower values as compared to their high-quality counterparts, no significant differences (p > 0.05) existed in most instances. Therefore, the combination of nutritional compounds together with functional characteristics renders combination effects that provide the characteristic quality of each grade of black tea.

AUTHOR INFORMATION

Corresponding Author

*E-mail: cesarettin.alasalvar@tubitak.gov.tr. Phone: +90 (0) 262 677 3272. Fax: +90 (0) 262 641 2309.

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The authors declare no competing financial interest.

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