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Flavor Characteristics of Seven Grades of Black Tea Produced in Turkey

Cesarettin Alasalvar,*[,†] Bahar Topal,[†] Arda Serpen,[‡] Banu Bahar,[†] Ebru Pelvan,[†] and Vural Gökmen[‡]

[†]TÜBİTAK Marmara Research Center, Food Institute, P.O. Box 21, 41470 Gebze-Kocaeli, Turkey

[‡]Department of Food Engineering, Hacettepe University, Beytepe-Ankara, 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 compared for their differences in descriptive sensory analysis (DSA), aroma-active compounds (volatile compounds), and taste-active compounds (sugar, organic acid, and free amino acid compositions). Ten flavor attributes such as 'after taste', 'astringency', 'bitter', 'caramel-like', 'floral/sweet', 'green/grassy', 'hay-like', 'malty', 'roasty', and 'seaweed' were identified. Intensities for a number of flavor attributes ('after taste', 'caramel-like', 'malty', and 'seaweed') were not significantly different ($p > 0.05$) among seven grades of black tea. A total of 57 compounds in seven grades of black tea (14 aldehydes, eight alcohols, eight ketones, two esters, four aromatic hydrocarbons, five aliphatic hydrocarbons, nine terpenes, two pyrazines, one furan, two acids, and two miscellaneous compounds) were tentatively identified. Of these, aldeyhdes comprised more than 50% to the total volatile compounds identified. In general, high-grade quality tea had more volatiles than low-grade quality tea. With respect to taste-active compounds, five sugars, six organic acids, and 18 free amino acids were positively identified in seven grades of black tea, of which fructose, tannic acid, and theanine predominated, respectively. Some variations ($p < 0.05$), albeit to different extents, were observed among volatile compounds, sugars, organic acids, and free amino acids in seven grades of black tea. The present study suggests that a certain flavor attributes correlate well with taste- and aroma-active compounds. High- and low-quality black teas should not be distinguished solely on the basis of their DSA and taste- and aroma-active compounds. The combination of taste-active compounds together with aroma-active compounds renders combination effects that provide the characteristic flavor of each grade of black tea.

KEYWORDS: *grades, black tea, aroma-active compounds, taste-active compounds, volatile aroma compounds, flavor attributes, descriptive sensory analysis*

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 that of 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 the production of 198,601 MT.[1]

The original purpose of tea fermentation was to enhance the flavor of tea. Therefore, flavor is the most important element for tea evaluation.[2] Flavor comprises principally taste- and aroma-active compounds. Nonvolatile compounds (e.g., organic acids, sugars, and free amino acids, among others) are generally responsible for the taste, while volatile compounds (e.g., aldehydes, alcohols, ketones, furans, and aromatic compounds, among others) provide the aroma. Despite the fact that volatile compounds are present in minute amounts, these have a high impact on the flavor of the products due to their low threshold values and resulting in high odor units.[3]

Compounds responsible for taste in black tea are mainly polyphenols, free amino acids, caffeine, catechin, theaflavins, and thearubigins.[4,5] To date, around 600 volatile compounds have been reported in tea leaves or the beverages.[3,6−15] The 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.[16] Therefore, control of the fermentation process has important effects on the flavor and color of tea, which depends on the degree of oxidation of tea phenolics. On the one hand, 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".[5,17] On the other hand, thearubigins are black-brown or reddish-brown in color and contribute to the color, acidity, body, ashy, and slight astringency of tea.[5,18] Both taste- and aroma-active compounds give the characteristic flavor and color of black tea.

The common process of black tea production consists of four stages, namely, withering, rolling, fermentation, and firing. Besides being differentiated by their origin, three main types of tea are generally produced: green (unfermented), oolong (semifermented), and black (fermented).[13] Black tea is processed in either of two ways: Orthodox or CTC (Crush, Tear, and Curl).[19,20] It is usually graded on one of four scales of quality (such as whole leaf, broken leaf, fannings, and dust). In Turkey, more than 50% of black tea is processed by ÇAYKUR Tea Processing Plant, which processes black tea according to its own seven different grades [high-quality tea (grades 1−3) and

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low-quality tea (grades 4−7)] (Table 1). The low-quality groups of teas are marketed after being blended with high-quality group categories according to the demands.

Table 1. Seven Grades of Black Tea According to ÇAYKUR Processings

quality	grade	acronmy	description
high-quality black tea	grade 1	OF	Orange Fannings
	grade 2	BOP-1	Broken Orange Pekoe-1
	grade 3	OP	Orange Pekoe
low-quality black tea	grade 4	F	Fannings
	grade 5	BOP-2	Broken Orange Pekoe-2
	grade 6	BP	Broken Pekoe
	grade 7	FD	Fine Dust

With respect to the flavor and sensory characteristics, there are no data available in the literature to distinguish the seven grades of black tea produces in Turkey. Thus, it is important to define both the taste- and the aroma-active compounds in different grades of Turkish-grown tea as such data may lead to the improvement of the quality of products in the market and could also increase consumption. The objective of this study was to compare taste- and aroma-active compounds as well as flavor attributes of seven grades of black tea produced in Turkey.

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 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 chemicals used were obtained from Sigma-Aldrich-Fluka Company Ltd. (Prolab, Istanbul, Turkey), unless otherwise stated. All standards and reference materials used for training of panelists were food grade. High-purity (>98%) free amino acid standards were purchased from Merck Corporate (Darmstadt, Germany).

Descriptive Sensory Analysis (DSA). Seven grades of black tea were assessed using flavor profile method of ISO 6564.[21] Prior to DSA, panelists discussed the flavor properties of Turkish black tea samples during the three preliminary orientation sessions, each lasting 90 min, until they had agreed on their use as flavor attributes. During these orientation sessions, panelists evaluated six different brands of black tea (Altınbaş, Çay Çiçeği, Filiz, Kamelya, Rize Turist, and Tiryaki) produced by ÇAYKUR Tea Processing Plant (Rize, Turkey). Fifteen flavor attributes by observing odor, taste, and mouthfeel were tested, 10 of which were selected by panelists with a consensus agreement that most describes the black tea. The flavor attributes together with their definitions, standards, and reference materials used are given in Table 2. Standards and reference materials used were carried out according to Schuh and Schieberle.[13]

A 2.85 g of black tea was infused with 140 mL of freshly boiled water for 6 min in a special tea pot according to ISO 3103.[63] DSA was employed for evaluation of the black tea samples, using a 10 cm long line anchors of 0 = none and 10 = very, by 10 well-trained panelists (four males and six females, aged 24−44 years). The evaluation was carried out using the computerized testing booths, where EyeQuestion software (version 2.4, Elst, The Netherlands) was used. Each grade of black tea was randomly evaluated twice on different days. Data are expressed as means ± standard deviations (SDs) (n = 10 well-trained panelists).

Preparation of Internal Standard (IS) and Deodorized Water for Volatile Compounds. The IS (5-methyl-2-hepten-4-one) was dissolved in high-performance liquid chromatography (HPLC) grade methanol at a concentration of 1000 ppm. The final concentration of 25 ppm was prepared by dilution in deodorized water to fully hydrate the tea sample for proper flavor release. HPLC-grade water and filtered water gave many artifacts on the chromatograph. Instead, deodorized water prepared daily was used. For this, HPLC-grade water was boiled in an open flask until its volume was decreased by one-thirds of the original. The flask was covered with aluminum foil after boiling and during cooling.

Headspace/Gas Chromatography/Mass Spectrometry (HS/GC/MS). Volatile compounds in tea were analyzed by HS/GC/MS. Total ion chromatograms of volatiles were obtained using a Perkin-Elmer HS 40 and Clarus 600 C GC/MS (PerkinElmer Inc., Oak Brook, IL). They were measured according to the procedure described by Alasalvar et al.,[22−24] with a slight modification.

A 1 g tea sample was transferred into a 22 mL headspace vial (PerkinElmer Inc., Oak Brook, IL), and a 100 μL aliquot of the 25 ppm aqueous IS solution (5-methyl-2-hepten-4-one) was added. The

Table 2. Flavor Attributes with Definitions, Standards, and Reference Materials Used for DSA[a]

attribute	sensory attribute definitions	standards and reference materials
astringency	feeling of dryness in mouth	grape juice or four black tea bags in 1000 mL of boiled water infused for 5 min
bitter taste	bitter taste felt in back side of the tongue	1 g/L caffeine solvent
caramel-like/cotton candy flavor	caramel flavor associated with burned sugar (usually found in some black and oolong teas)	10^{-3} g/L 4-hydroxy-2,5 dimethyl-3(2H) furanone
cucumber flavor	odor associated with fresh cut cucumber	10^{-3} g/L (*E*,*Z*)-2,6-nonadienal
fishy/seaweed flavor	desired seaweed flavor occurred during green tea processing	1.75 g of green tea powder solved in 450 mL of boiled water
floral/citrus flavor	floral odors composed in tea leaves naturally or during processing	5×10^{-4} g/L (*R*)-linalool
fried flavor	sunflower flavor used in fried potato	10^{-3} g/L (*E*,*E*)-2,4-nonadienal
grassy odor	odor of fresh cut grass/plants	10^{-3} g/L hexanal
green odor	odor of green plants	10^{-3} g/L phenylacetaldehyde
honey-like/sweet taste	pleasant flavor of honey or sugar left in front of the tongue	10^{-3} g/L(*E*)-β-damascenone
malty flavor	flavor associated with germinated malt	10^{-3} g/L 3-methylbutanal
oily	sunflower oil flavor	10^{-3} g/L (*E*,*E*)-2,4-decadienal
rose odor and taste	rose-like	5×10^{-4} g/L geraniol
sour taste	puckering up of mouth	1.5 g/L citric acid
umami taste	flavor associated with umami	0.6 g/L umami

[a]Flavor attributes were selected by observing odor, taste, and mouthfeel of seven different coded black teas. Only astringency, bitter, sour, and umami flavor attributes were tasted.

Table 3. DSA of Flavor Attributes in Seven Grades of Black Tea (Scaling: 0 = None, 10 = Very)[a]

attributes	grade 1	grade 2	grade 3	grade 4	grade 5	grade 6	grade 7
after taste	5.0 ± 1.3 a	4.9 ± 1.0 a	5.4 ± 1.4 a	4.8 ± 1.0 a	4.4 ± 0.9 a	5.5 ± 1.7 a	5.5 ± 1.6 a
astringency	4.1 ± 1.5 ab	3.9 ± 1.1 ab	4.6 ± 0.6 ab	4.0 ± 0.7 ab	3.7 ± 1.1 ab	2.9 ± 1.1 a	5.2 ± 0.9 b
bitter	4.6 ± 0.7 ab	3.4 ± 1.1 a	4.0 ± 1.2 ab	4.4 ± 1.1 ab	3.5 ± 1.4 a	2.9 ± 0.9 a	5.8 ± 1.1 b
caramel-like	1.5 ± 0.6 a	1.7 ± 0.6 a	1.4 ± 0.5 a	2.0 ± 0.6 a	1.6 ± 0.3 a	1.7 ± 0.9 a	1.7 ± 0.6 a
floral/sweet	2.7 ± 1.2 ab	5.5 ± 1.1 c	4.6 ± 1.1 bc	3.8 ± 1.0 abc	4.0 ± 1.0 abc	3.4 ± 0.9 abc	2.5 ± 0.9 a
green/grassy	2.6 ± 0.9 a	6.1 ± 1.6 c	4.7 ± 0.8 bc	3.1 ± 1.3 ab	3.4 ± 1.2 abc	3.4 ± 1.1 abc	2.8 ± 1.0 ab
hay-like	5.6 ± 0.8 ab	3.5 ± 1.5 a	4.2 ± 0.8 ab	5.3 ± 1.3 ab	4.6 ± 1.3 ab	5.4 ± 0.8 ab	5.8 ± 1.2 b
malty	3.2 ± 1.7 a	2.0 ± 1.0 a	2.1 ± 1.0 a	3.3 ± 1.8 a	4.6 ± 1.5 a	2.1 ± 1.1 a	3.1 ± 1.5 a
roasty	4.0 ± 1.2 ab	3.5 ± 0.8 a	3.4 ± 1.2 a	4.2 ± 0.8 ab	5.1 ± 1.0 ab	5.1 ± 1.6 ab	5.8 ± 1.0 b
seaweed	2.7 ± 1.2 a	2.5 ± 1.2 a	2.7 ± 0.7 a	2.2 ± 0.6 a	2.3 ± 0.6 a	3.4 ± 1.4 a	3.6 ± 1.1 a

[a]Data are expressed as means ± SDs (n = 10 well-trained panelists). Means ± SDs followed by the same letter, within a row, are not significantly different ($p > 0.05$).

headspace vial was immediately sealed (with cramp top aluminum caps) and mixed for ~10 s using a Vortex prior to analysis. The sample was then heated at 80 °C for 60 min. The vial and column pressures were set at 10 and 25 psi, respectively. The needle and transfer line temperatures were 90 and 100 °C, respectively. The trap hold time was 6 min, and the outlet split was on. Desorbed compounds were automatically injected into a GC column (DB Wax, 60 m × 0.25 mm i.d. × 0.25 μm film thickness; Agilent Technologies Inc., Santa Clara, CA). The flow rate of the helium carrier gas was 1 mL/min. Each sample was injected in the splitless mode (200 °C injection port temperature). The GC column temperature was programmed from initial holding at 35 °C for 5 min and then from 35 to 160 °C at a rate of 3 °C/min.

MS conditions were as follows: ion source temperature, 180 °C; ionization energy, 70 eV; mass scan range, 33−300 a.m.u.; electron multiplier voltage, 450 V; scan speed, 1188 Da/s; and ion mode, electron ionization (EI). All analyses were performed in triplicate for each tea sample, and the results were averaged.

Compound Identification and Relative Amounts. Positive identifications were based on comparison of GC retention indices (RI), determined using n-alkanes (C_8−C_{15})[25] and mass spectra of unknowns with those of authentic standard compounds analyzed under identical experimental conditions. Tentative identifications were based on matching mass spectra of unknowns with those in the NIST mass spectral database. The relative concentration of a compound in tea sample was calculated as follows:

$$\text{relative concentration (ng/g)} = \frac{(\text{peak area of unknown compound/peak area of IS}) \times 2500\ \text{ng of IS}}{\text{amount of tea (1 g)}}$$

Sugar Analysis. Sugar levels were measured according to the HPLC method of Serpen and Gökmen[26] with some modifications. A 1 g tea sample was extracted with 8 mL of hot water (80 °C) for 10 min, together with 1 mL of both Carrez 1 (15 g of potassium hexacyanoferrate in 100 mL of water) and Carrez 2 (30 g of zinc sulfate in 100 mL of water). The supernatant was collected into a flask after centrifugation at 7500g for 5 min. The residue was further extracted with 5 mL of hot water (80 °C) for two times. One milliliter from combined extract was passed through an Oasis HLB cartridge, preconditioned with 1 mL of methanol and 1 mL of HPLC-grade water, to clean up the extract. The first eight drops of the eluent were discarded, and the rest was collected into a HPLC vial. Chromatographic analyses were performed on an Agilent 1200 HPLC system consisting of a refractive index (RI) detector, quaternary pump, autosampler, and column oven. An isocratic elution with deionized water at a flow rate of 0.4 mL/min was used. A 10 μL of sample was injected into a Transgenomic Carbosep CHO-682 column (Transgenomic, Glasgow, United Kingdom), 200 mm × 7.8 mm, at 20 °C. Identified sugars were quantified on the basis of peak areas and comparison with a calibration curve obtained with the corresponding standards.

Organic Acid Analysis. Organic acids were extracted according to the method of Serpen and Gökmen.[26] A 1 g 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 7500g for 5 min. The residue was further extracted with 5 mL of hot water (80 °C) for two times. The combined extract was diluted with water at a final ratio of 1 g of black tea in 50 mL. One milliliter of diluted extract was passed through a preconditioned Oasis HLB cartridge. The first eight drops of the eluent were discarded, and the rest was collected into an HPLC vial. Chromatographic analyses were performed on an Agilent 1200 HPLC system consisting of a diode array detector (DAD), quaternary pump, autosampler, and column oven. An isocratic elution with a mobile phase consisting 0.1% formic acid in water (v/v) at a flow rate of 1 mL/min was used. A 10 μL of sample was injected into a Shodex Rspak KC-811 column, 300 mm × 7.8 mm (Shoko Co., Tokyo, Japan), at 20 °C. Chromatograms were recorded at 220 nm with spectra (200−700 nm) taken continuously throughout the elution. Identification of organic acids was accomplished by comparing the retention time and absorption spectra of peaks in tea samples to those of standard compounds. The quantitation of organic acids was based on calibration curves built for each of the compounds identified in tea samples.

Free Amino Acid Analysis. A 1 g 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 7500g for 5 min. The residue was further extracted with 5 mL of hot water (80 °C) for two times. The combined extract was diluted five-fold with a final ratio of water:acetonitrile (50:50, v/v). Finally, the extract was filtered through 0.45 μm nylon filter and transferred into HPLC vial. Analysis of free amino acids was accomplished with hydrophilic interaction liquid chromatography coupled to high-resolution Orbitrap Mass Spectrometry based on the method described by Gökmen et al.[27]

Statistical Analysis. The statistical significance of the sensory attributes of seven grades of black tea was evaluated by nonparametric test with 5% significance level (Kruskal−Wallis, XLSTAT, version 2009/05/01; Addinsoft, Paris, France). Differences were considered to be significant at $p \leq 0.05$. Differences for other analyses were estimated by analysis of variance (ANOVA) followed by Tukey's "honest significant difference" test. Statistical analyses were performed using the SPSS 18.0 version (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Sensory Evaluation. Table 3 shows the DSA of flavor attributes in seven grades of black tea ('after taste', 'astringency', 'bitter', 'caramel-like', 'floral/sweet', 'green/grassy', 'hay-like', 'malty', 'roasty', and 'seaweed'). These flavor attributes were detected in all grades of black tea, albeit to different extents. Intensities for a number of flavor attributes ('after taste', 'caramel-like', 'malty', and 'seaweed') were not significantly different ($p > 0.05$) among seven grades of black tea. No significant differences ($p > 0.05$) in 10 flavor attributes were observed among high-quality black tea (grades 1−3), except for 'floral/sweet' and 'green/grassy', which were significat ($p < 0.05$). In general, grade 2 had the highest intensities of 'floral/sweet' and 'green/grassy' (Table 3).

With respect to low-grade quality black tea (grades 4−7), no significant differences ($p > 0.05$) were observed among 10 flavor attributes, except for 'astringency' and 'bitter', which were significant ($p < 0.05$). The intensities of 'after taste', 'astringency', 'bitter', 'roasty', and 'woody' were predominant in grade 7. This was due to double roasting of low-grade quality black tea and fine dust.

Schuh and Schieberle[13] identified nine flavor attributes from a Darjeeling Gold Selection of black tea infusion ('caramel-like', 'citrus/fruity', 'fatty', 'fishy', 'green/grassy', 'hay-like', 'malty', 'oatmeal flakes-like/sweet', and 'rose-like/honey-like'). Among them, 'caramel-like', 'green/grassy', 'hay-like', 'malty', and 'sweet' flavor attributes were detected in the present study. Kovács et al.[28] measured five Sri Lanka black teas from plantations of different geographical origins using sensory profile analysis. They identified 10 descriptors including color intensity (by untrained panelists), among which 'after taste', 'floral', 'hay-like', 'sweet', and 'bitter' were the same as the present study. Sensory characteristics of black tea also have been reported by others.[29−33] Several factors such as environmental factors, processing conditions, and part of tea leave used, among others, affect the sensory characteristics of black tea.

Certain volatile compounds identified in the present study are responsible for the sensory characteristics of black tea. For example, 2-methylypropanal, 2- and 3-methylbutanal (malty), hexanal, (*Z*)-2-penten-1-ol, (*Z*)-3-hexen-1-ol (green/grassy), linalool (floral), 2,3,5-trimethylpyrazine, 3-ethyl-2,5-dimethylpyrazine (roasty), and 2-pentylfuran (bitter and sweet).[12−15] The following taste-active compounds also impart sensory characteristic of black tea such as sugars (sweet),[34] tannic acid (astringency),[35,36] theanin, and glutamate (umami and sweet).[37−39] In addition to the above compounds, theaflavins and thearubigins are associated with astringency taste in black tea.[5,17] As a parallel of this study, flavanols including theaflavins and thearubigins were studied and will be published elsewhere.

Volatile Compounds. Qualitative and quantitative differences among seven grades of black tea were observed (Table 4). This might result from different processing methods and parts of tea leaves used. A total of 57 compounds in seven grades of black tea (14 aldehydes, eight alcohols, eight ketones, two esters, four aromatic hydrocarbons, five aliphatic hydrocarbons, nine terpenes, two pyrazines, one furan, two acids, and two miscellaneous compounds) were tentatively identified. In addition, 24 compounds remained unidentified. The total content of unknown compounds ranged from 24.1% (in grade 3) to 30.4% (in grade 1) of the total volatile present among seven grades of black tea. The contribution of volatiles to flavor is dependent upon their recognition threshold values and concentrations. Total relative concentrations of volatiles among seven grades of black tea ranged from 188,384 to 239,725 ng/g, being lowest in grade 6 and highest in grade 5. The number of compounds detected ranged from 38 in grade 7 to 57 in grade 1. In general, high-quality tea (grades 1−3) had more volatiles than low-quality tea (grades 4−7). In other words, there was a decreasing trend in number of volatiles detected as grades lower (Table 4). Although the selection of the "best" grade is a subjective matter, the combination of several classes of aroma-active compounds is responsible for the distinctive and unique flavor of different grades of tea.

Aldehydes. Fourteen aldehydes were found in seven grades of black tea. They comprised more than 50% to the total volatile compounds identified (Table 4). Among them, the concentrations of 2-methylypropanal, 2- and 3-methylbutanal, hexanal, and (*E*)-2-hexenal were much higher than other aldehydes detected. These compounds have been previously identified in black tea infusion[13] and green tea.[11] 2-Methylypropanal and 2- and 3-methylbutanal have been reported to be responsible for a malty smell in black tea infusion,[13] whereas hexanal and (*E*)-2-hexenal possess a green and grassy odor in black tea leaves[13] and a fragrant, sweet, and fruity odor in oolong tea,[14] respectively. Wang et al.[15] reported that the main aldehyde constituents in black tea were hexanal, (*E*)-2-hexenal, benzaldehyde, and benzeneacetaldehyde. Except benzeneacetaldehyde, all compounds were detected in the present study. The hexanal concentration has been observed to increase after fermentation.[14] In addition, (*E,E,Z*)-2,4,6-nonatrienal has been reported in black tea infusion, as a character impact odorant (oat-flake-like).[13] This compound was not detected in the present study. The majority of aldehydes, which contribute green, malty, fatty, sweet, floral, and fruity aromas in foods, are generally considered lipid autoxidation products during manufacture.[40,41] It was found that the fermentation process can, but not necessarily, cause the loss of grassy or green odors, whereas formation of the fruity/floral and other fermented characters increases in black tea.[14,15]

Alcohols. Eight alcohols were identified, among which (*Z*)-2-penten-1-ol and (*Z*)-2-hexen-1-ol were the most abundant (Table 4). The eight alcohols detected in the present study, except benzyl alcohol, have been reported in green tea.[11] In addition, (*Z*)-2-penten-1-ol and (*Z*)-3-hexen-1-ol were detected in various types of tea.[13,15] (*Z*)-3-Hexen-1-ol has been reported as being the most abundant alcohol detected in various black teas from different regions of India.[4] (*Z*)-2-Penten-1-ol is known to possess fresh, green, and metallic odors in oolong tea,[14] and (*Z*)-3-hexen-1-ol is known to have green odor in black tea leaves[13] and different green tea varieties.[12] Benzyl alcohol, which imparts a mild sweet and roasted odor in oolong tea, has been reported in various types of tea.[4,14,15] Volatile alcohols are generally minor contributors to food flavor because of their high thresholds unless they are present at high concentrations or are unsaturated.[42] The majority of the other alcohols detected may be formed by the decomposition of hydroperoxides of fatty acids[43] or by reduction of aldehydes. The relative concentration of total alcohols varied between 7121 ng/g in grade 1 and 8325 ng/g in grade 2.

Ketones. Eight ketones were found in seven grades of black tea (Table 4). The most abundant compounds among identified ketones were 2,3-butanedione and 1-penten-3-one. Most ketones found in present study were also reported in various types of tea.[10−15] It has been reported that 2,3-butanedione imparts a buttery odor in black tea leaves[13] and green tea varieties,[12] while 1-octen-3-one imparts mushroomlike odor in black tea leaves.[13] In addition, odor descriptions of 1-penten-3-one (harsh and pungent) and 6-methyl-5-hepten-2-one (herbaceous, pungent, and oil) have been reported in oolong tea.[14] Generally, because of their overall low aroma threshold values,[44] ketones play a significant contribution to overall aroma of tea. Ketones may be produced by thermal oxidation/degradation of polyunsaturated fatty acids,[44] amino acid degradation,[45] or microbial spoilage.[46] Ketones, which were the second most abundant compounds after aldehydes in seven grades of black tea, ranged from 13,598 ng/g in grade 3 to 22,039 ng/g in grade 5 in total.

Esters. Two esters, namely, methyl butanoate and butyl acetate, were detected in seven grades of black tea (Table 4). These two compounds have been reported in black and green teas.[10,11] Benzyl acetate, which was not detected in the present study, was previously reported in various types of tea and possesses a floral, fruity, sweet, and fresh odor in oolong tea.[14]

Table 4. Volatile Compounds in Seven Grades of Black Tea (ng/g)[a]

compd name by class	MI[b]	RI[c]	grade 1	grade 2	grade 3	grade 4	grade 5	grade 6	grade 7
					aldehydes				
2-methylpropanal	MS[d]	740	67156 ± 4319 a	55489 ± 1575 a	57947 ± 6650 a	64343 ± 16348 a	69687 ± 4508 a	49833 ± 26794 a	61779 ± 6652 a
2-methylbutanal	MS, RI	914	14159 ± 636 a	7936 ± 5904 a	12255 ± 1291 a	13990 ± 2347 a	14884 ± 1190 a	11656 ± 3490 a	13369 ± 907 a
3-methylbutanal	MS, RI	918	13923 ± 743 ab	11192 ± 57 ab	12478 ± 1529 ab	14135 ± 1843 ab	15567 ± 426 a	13187 ± 3047 ab	10793 ± 1265 b
hexanal	MS, RI	1084	10457 ± 730 a	12173 ± 363 a	12023 ± 1536 a	13347 ± 329 a	14693 ± 2055 a	12741 ± 3453 a	12375 ± 1004 a
2-methyl-2-butenal	RI	1098	214 ± 188 a	255 ± 12 a	nd a	nd a	nd a	253 ± 438 a	1922 ± 160 b
(*E*)-2-pentenal	MS, RI	1123	220 ± 75 a	21 ± 36 a	168 ± 146 a	183 ± 126 a	84 ± 145 a	nd a	nd a
2-methyl-2-pentenal	MS, RI	1159	94 ± 110 a	22 ± 38 a	77 ± 33 a	81 ± 140 a	102 ± 177 a	100 ± 39 a	nd a
heptanal	RI	1187	489 ± 140 a	354 ± 13 a	365 ± 110 a	418 ± 125 a	411 ± 100 a	205 ± 179 a	455 ± 36 a
(*Z*)-3-hexenal	MS, RI	1214	90 ± 41 a	108 ± 4 a	80 ± 32 a	57 ± 52 a	103 ± 28 a	100 ± 15 a	99 ± 9 a
(*E*)-2-hexenal	MS, RI	1223	9424 ± 360 a	8514 ± 229 ab	9044 ± 1228 ab	9251 ± 1659 ab	8474 ± 985 ab	8564 ± 534 ab	6721 ± 820 b
(*E*)-2-heptenal	RI	1294	13 ± 22 a	nd a	48 ± 84 a	nd a	nd a	nd a	nd a
nonanal	RI	1397	104 ± 90 a	nd b	nd b	nd b	nd b	nd b	nd b
benzaldehyde	MS, RI	1536	587 ± 109 a	622 ± 27 a	693 ± 64 ab	713 ± 128 ab	712 ± 73 ab	708 ± 94 ab	885 ± 113 b
(*E*)-2-nonenal	RI	1539	12 ± 21 a	nd a	nd a	nd a	nd a	nd a	nd a
					alcohols				
2-propanol	MS	905	306 ± 45 ab	357 ± 12 a	327 ± 67 ab	327 ± 72 ab	393 ± 30 b	277 ± 244 ab	787 ± 76 c
ethanol	RI	928	103 ± 44 a	108 ± 18 a	98 ± 16 a	28 ± 27 a	92 ± 34 a	23 ± 41 a	45 ± 27 a
2-methyl-1-propanol	RI	1089	20 ± 34 a	nd a	nd a	nd a	nd a	nd a	69 ± 120 a
(*Z*)-2-penten-1-ol	MS. RI	1332	1454 ± 443 a	2299 ± 19 a	1933 ± 358 a	1185 ± 169 a	1618 ± 1407 a	2260 ± 288 a	2114 ± 197 a
1-hexanol	RI	1365	460 ± 56 a	7 ± 11 b	229 ± 181 abc	407 ± 29 ac	442 ± 50 a	121 ± 210 bc	352 ± 41 ac
(*Z*)-3-hexen-1-ol	MS, RI	1396	4263 ± 881 a	5072 ± 90 a	4221 ± 727 a	4204 ± 535 a	4579 ± 549 a	4404 ± 275 a	4382 ± 845 a
3-ethylhexan-1-ol	RI	1474	168 ± 45 a	121 ± 7 ab	nd c	105 ± 39 ab	nd c	62 ± 55 bc	nd c
benzyl alcohol	MS	nm[g]	349 ± 322 a	362 ± 314 a	387 ± 199 a	269 ± 180 a	378 ± 189 a	385 ± 70 a	499 ± 176 a
					ketones				
2,3-butanedione	RI	953	13580 ± 749 a	14901 ± 215 a	10400 ± 9125 a	17194 ± 2023 a	18550 ± 915 a	15168 ± 4379 a	14492 ± 1753 a
1-pentene-3-one	MS, RI	1020	1671 ± 96 ab	1626 ± 80 ab	2581 ± 297 a	2240 ± 220 ab	3046 ± 380 a	2074 ± 1498 ab	648 ± 81 b
2,3-pentanedione	RI	1062	67 ± 13 a	59 ± 13 a	68 ± 28 a	41 ± 37 a	nd a	18 ± 3 a	150 ± 193 a
4-methyl-3-penten-2-one	RI	1131	71 ± 87 a	nd a	252 ± 375 a	574 ± 740 a	83 ± 143 a	nd a	nd a
3-hydroxy-2-butanone	RI	1286	80 ± 139 a	nd a	nd a	nd a	nd a	73 ± 68 a	nd a
1-octen-3-one	RI	1305	79 ± 47 ab	40 ± 35 a	101 ± 14 ab	151 ± 42 b	128 ± 29 ab	167 ± 67 b	78 ± 10 ab
6-methyl-5-hepten-2-one	MS, RI	1345	200 ± 17 a	266 ± 8 a	196 ± 50 a	219 ± 35 a	233 ± 4 a	227 ± 5 a	381 ± 40 b
4-hydroxy-4-methylpentan-2-one	RI	1347	33 ± 8 a	nd b	nd b	nd b	nd b	nd b	nd b
					esters				
methyl butanoate	RI	971	664 ± 23 ab	626 ± 21 ab	630 ± 69 ab	733 ± 246 ab	741 ± 68 ab	478 ± 240 a	900 ± 169 b
butyl acetate	RI	1050	111 ± 47 a	170 ± 15 a	108 ± 36 a	85 ± 86 a	164 ± 56 a	201 ± 51 a	221 ± 191 a
					aromatic hydrocarbons				
benzene	RI	940	169 ± 22 a	168 ± 12 a	164 ± 22 a	192 ± 29 a	218 ± 8 a	232 ± 58 a	408 ± 77 b
toluene	MS, RI	1040	325 ± 312 ab	652 ± 15 a	586 ± 41 a	488 ± 256 ab	583 ± 120 a	360 ± 181 ab	51 ± 5 b
1-methyl-3-ethylbenzene	RI	1219	30 ± 37 a	7 ± 12 a	nd a	nd a	nd a	7 ± 12 a	5 ± 8 a
1-methyl-2-propylbenzene	RI	1329	6 ± 11 a	nd a	nd a	nd a	576 ± 998 a	nd a	nd a

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Table 4. continued

compd name by class	MI[b]	RI[c]	grade 1	grade 2	grade 3	grade 4	grade 5	grade 6	grade 7
					aliphatic hydrocarbons				
n-decane	RI	1000	143 ± 1 a	180 ± 32 ab	197 ± 15 abc	165 ± 18 ab	237 ± 29 bc	185 ± 47 ab	259 ± 12 c
n-undecane	RI	1101	966 ± 1254 ab	293 ± 255 ab	2032 ± 1190 ab	1049 ± 1192 ab	3025 ± 334 a	1991 ± 1609 ab	65 ± 68 b
n-butane	RI	1137	142 ± 123 a	453 ± 549 a	117 ± 128 a	170 ± 161 a	84 ± 146 a	761 ± 911 a	368 ± 274 a
2,5-dimethylhexene	RI	1198	48 ± 43 a	nd a	17 ± 29 a	11 ± 19 a	15 ± 26 a	nd a	nd a
3-methyltridecane	RI	1368	33 ± 29 a	544 ± 38 b	156 ± 270 ab	nd a	nd a	296 ± 258 ab	nd a
					terpenes				
δ-3-carene	RI	1141	632 ± 578 ab	169 ± 147 a	461 ± 399 ab	374 ± 330 a	1468 ± 472 b	267 ± 408 a	nd a
α-phellandrene	RI	1161	100 ± 113 ab	65 ± 70 ab	18 ± 30 a	69 ± 120 ab	nd a	17 ± 29 a	224 ± 33 b
α-terpinene	RI	1172	5463 ± 779 a	7880 ± 292 a	7365 ± 1095 a	8102 ± 1176 a	8990 ± 901 a	6168 ± 5335 a	8387 ± 774 a
limonene	RI	1194	2617 ± 2266 abc	5662 ± 141 a	4022 ± 193 ab	2969 ± 266 abc	3303 ± 172 ab	2188 ± 1895 bc	nd c
d-limonene	RI	1203	23 ± 20 a	nd b	nd b	nd b	nd b	nd b	nd b
p-cymene	RI	1277	567 ± 982 a	14 ± 24 a	nd a	nd a	nd a	nd a	nd a
camphor	RI	1504	135 ± 31 a	116 ± 1 a	86 ± 18 a	97 ± 13 a	102 ± 17 a	67 ± 58 a	145 ± 23 a
(*Z*)-linalool oxide	MS, RI	1485	129 ± 122 a	241 ± 20 a	166 ± 116 a	149 ± 75 a	216 ± 115 a	196 ± 46 a	70 ± 62 a
linalool	MS, RI	1552	28 ± 26 a	nd a	nd a	nd a	nd a	133 ± 231 a	nd a
					pyrazines				
2,3,5-trimethylpyrazine	RI	1418	782 ± 160 a	877 ± 83 a	762 ± 189 a	785 ± 125 a	923 ± 196 a	850 ± 191 a	720 ± 63 a
3-ethyl-2,5-dimethylpyrazine	RI	1439	28 ± 48 a	47 ± 43 a	nd a	nd a	nd a	nd a	nd a
					furan				
2-pentylfuran	MS, RI	1232	531 ± 462 a	988 ± 69 a	1082 ± 47 a	1185 ± 168 a	1469 ± 95 a	986 ± 854 a	1051 ± 82 a
					acids				
3-hexanoic acid	MS	nm	434 ± 301 a	78 ± 69 b	60 ± 64 b	37 ± 64 b	77 ± 68 b	90 ± 10 ab	nd b
acetic acid	MS, RI	1463	3747 ± 1355 a	4209 ± 337 a	3247 ± 1604 a	3571 ± 1289 a	4254 ± 1120 a	3493 ± 616 a	4906 ± 580 a
					miscellaneous				
benzeneacetamide	MS	nm	132 ± 122 a	72 ± 12 a	116 ± 62 a	42 ± 73 a	93 ± 82 a	121 ± 21 a	184 ± 26 a
2-acetyl-1-pyrroline	RI	1342	24 ± 24 a	41 ± 3 a	15 ± 26 a	13 ± 22 a	24 ± 22 a	25 ± 22 a	36 ± 7 a
5-methyl-2-hepten-4-one (IS)[e]		1298							
total unknowns			69080 (30.4%)[f]	48818 (25.1%)	46759 (24.1%)	52518 (24.2%)	58904 (24.6%)	46851 (24.9%)	53673 (26.3%)
total volatiles			226933 ± 47703 a	194374 ± 12819 a	194135 ± 38124 a	217229 ± 43263 a	239725 ± 27677 a	188384 ± 80497 a	204065 ± 23387 b

[a]Data are expressed as the means ± SDs ($n = 3$) on a fresh weight basis. For letters a−c, means ± SDs followed by the same letter, within a row, are not significantly different ($p > 0.05$). [b]MI, methods of identification. [c]RI, retention indices. [d]MS, mass spectral data. [e]IS, internal standard. [f]Numbers in parentheses indicate the percent of unknown compounds in the total amount of volatiles. [g]nm, not measured, beyond *n*-alkane (C17).

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Table 5. Sugar Compositions in Seven Grades of Black Tea (g/100 g)[a]

	grade 1	grade 2	grade 3	grade 4	grade 5	grade 6	grade 7
fructose	1.10 ± 0.01 d	1.02 ± 0.01 c	0.99 ± 0.01 b	1.16 ± 0.02 e	0.99 ± 0.02 b	0.91 ± 0.02 a	1.04 ± 0.01 c
galactose	0.08 ± 0.00 c	0.10 ± 0.00 e	0.08 ± 0.00 c	0.09 ± 0.00 d	0.08 ± 0.00 b	0.07 ± 0.00 a	0.09 ± 0.00 c
glucose	1.87 ± 0.03 f	1.08 ± 0.03 a	1.28 ± 0.03 c	1.58 ± 0.01 e	1.23 ± 0.03 bc	1.21 ± 0.01 b	1.47 ± 0.03 d
sucrose	0.50 ± 0.01 d	0.49 ± 0.01 d	0.46 ± 0.01 c	0.61 ± 0.01 e	0.29 ± 0.01 a	0.28 ± 0.01 a	0.32 ± 0.01 b
xylose	0.04 ± 0.00 c	0.01 ± 0.00 a	0.05 ± 0.00 e	0.04 ± 0.00 c	0.04 ± 0.00 d	0.04 ± 0.00 d	0.03 ± 0.00 b
total	3.59 ± 0.02 e	2.70 ± 0.03 b	2.86 ± 0.04 c	3.48 ± 0.04 d	2.63 ± 0.06 b	2.51 ± 0.04 a	2.95 ± 0.06 c

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

Table 6. Organic Acid Compositions in Seven Grades of Black Tea (mg/100 g)[a]

	grade 1	grade 2	grade 3	grade 4	grade 5	grade 6	grade 7
citric	283 ± 39 ab	318 ± 3 ab	305 ± 37 ab	278 ± 32 ab	306 ± 6 ab	265 ± 4 a	343 ± 40 b
fumaric	3.6 ± 0.4 b	4.0 ± 0.2 b	3.7 ± 0.3 b	3.3 ± 0.6 ab	4.1 ± 0.4 b	2.5 ± 0.3 a	3.8 ± 0.4 b
glucuronic	79 ± 8 ab	73 ± 11 ab	102 ± 13 b	52 ± 21 a	107 ± 23 b	79 ± 5 ab	64 ± 5 a
malic	661 ± 78 b	749 ± 24 b	677 ± 124 b	715 ± 136 b	760 ± 78 b	575 ± 46 a	718 ± 119 b
oxalic	338 ± 6 a	407 ± 16 ab	423 ± 57 ab	405 ± 50 ab	419 ± 24 ab	340 ± 24 a	461 ± 42 b
tannic	3511 ± 45 a	4261 ± 117 abc	4273 ± 609 abc	3765 ± 418 ab	4407 ± 247 bc	3660 ± 368 ab	4672 ± 219 c
total	4876 ± 635 a	5812 ± 138 abc	5784 ± 840 abc	5218 ± 615 abc	6003 ± 178 bc	4921 ± 439 ab	6262 ± 187 c

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

In addition, ethyl 2-methyl butanoate was reported to impart a fruity note in black tea leaves.[13]

Aromatic Hydrocarbons. Four aromatic hydrocarbons were detected, of which toluene was the most abundant and present in all grades of black tea (Table 4). Among aromatic hydrocarbons, only toluene has been previously reported in green tea[11] and in various green, oolong, and black teas.[14] Toluene contributed no detectable odor in oolong tea.[14] Hydrocarbons, in general, have no effect on flavor.

Aliphatic Hydrocarbons. Five aliphatic hydrocarbons were detected in seven grades of black tea (Table 4), of which *n*-decane, *n*-undecane, and 3-methyltridecane were reported in green tea.[11] In addition, *n*-decane was also reported in various types of tea.[14] Aliphatic hydrocarbons, in general, present in different types of foods.

Terpenes. Nine terpenes were detected, among which linalool,[3,4,12−15] (*Z*)-linalool oxide,[3,4] camphor,[10] and *d*-limonene[11] were identified in various types of tea. Despite the fact that α-terpinene and limonene were the most abound terpenes detected in seven grades of black tea (Table 4), linalool has been reported as being floral odor in green tea varieties[12] and oolong tea.[14] *cis*-Linalool oxide has been reported to possess a sweet floral, green, and fruity odor in oolong tea.[14] Among seven grades of black tea, only grade 1 and grade 6 contained linalool.

Acids. 3-Hexanoic acid and acetic acid were the only acids detected in seven grades of black tea (Table 4). Among them, acetic acid was the most abundant. These two acids have previously been reported in green tea.[11] Hexanoic acid, which was not detected in the present study, was previously reported in black tea[3] and black tea leaves to be responsible for a sweaty odor.[13]

Pyrazines. In the present study, two pyrazines (2,3,5-trimethylpyrazine and 3-ethyl-2,5-dimethylpyrazine) were found. 3-Ethyl-2,5-dimethylpyrazine was only detected in grades 1 and 2 (Table 4). 2,3,5-Trimethylpyrazine in different green tea varieties[12] and 3-ethyl-2,5-dimethylpyrazine in green tea[11] have been reported. Various other pyrazines, which were not detected in the present study, were also reported in various types of tea.[11,12,14] Pyrazines, in general, contribute desirable roasty, sweet, and nutty odors in heat-induced foods/beverages, including tea.[23] Pyrazines were reported to be formed by Maillard reaction through Strecker degradation from various nitrogen sources such as amino acids.[47,48] Their contribution to overall aroma of tea is minor.

Furan. Only one furan (2-pentylfuran) was found in the present study (Table 4) and reported in various types of tea.[3,11,15] Furans arise from amino acids and sugars through Maillard and Strecker degradation reactions.[49] Furans, in general, contribute a significant aroma (burnt, sweet, bitter, cooked, meat, and coconut-like flavor) in some roasted foods.

Miscellaneous Compounds. Two miscellaneous compounds were found, of which 2-acetyl-1-pyrroline was reported in different green tea varieties and responsible for a popcorn-like odor.[12]

Taste-Active Compounds. Taste-active compounds such as sugars, organic acids, and free amino acids were studied.

Sugars. Five sugars were positively identified in seven grades of black tea; these included fructose, galactose, glucose, sucrose, and xylose. The total sugar content among seven grades of black tea ranged from 2.51 to 3.59 g/100 g, being lowest in grade 6 and highest in grade 1 (Table 5). Among identified sugars, glucose represented about 40−52% of the total amount, followed by fructose at 31−38% and sucrose at 11−18%. Other sugars (galactose and xylose) were present in small amounts. Significant differences (p < 0.05) existed among tea grades, with some exceptions. Sugars are responsible for sweetness of foods. Individual sugars possess different relative sweetness scores; fructose has been reported to be the sweetest sugar (sweetness score, 1.1−1.8), followed by sucrose (sweetness score, 1.0) and glucose (sweetness score, 0.5−0.8).[34]

Organic Acids. Six organic acids (citric, fumaric, glucuronic, malic, oxalic, and tannic) were positively identified in seven grades of black tea (Table 6). The organic acid content differed among seven grades of black tea. The total organic acid content varied between 4876 mg/100 g for grade 1 and 6262 mg/100 g for grade 7. Among the identified organic acids, tannic acid was most the abundant in all grades of tea, representing 73−75% to

Table 7. Free Amino Acid Compositions in Seven Grades of Black Tea (mg/100 g)[a]

	grade 1	grade 2	grade 3	grade 4	grade 5	grade 6	grade 7
alanine	131.2 ± 6.4 c	116.9 ± 6.1 abc	107.4 ± 4.1 ab	112.0 ± 4.8 ab	118.7 ± 9.5 abc	120.3 ± 7.3 bc	103.5 ± 2.7 a
arginine	73.5 ± 4.9 c	85.7 ± 0.8 d	80.6 ± 1.2 d	67.2 ± 0.9 b	85.8 ± 4.3 d	65.8 ± 0.4 b	48.4 ± 0.7 a
asparagine	87.9 ± 0.9 ab	84.8 ± 2.5 c	86.1 ± 3.5 c	87.0 ± 2.7 abc	91.7 ± 1.6 c	83.2 ± 1.1 bc	92.1 ± 0.7 a
aspartic acid	85.3 ± 0.9 b	87.3 ± 1.5 b	79.8 ± 1.2 a	85.8 ± 0.7 b	82.9 ± 3.4 ab	84.0 ± 1.6 b	84.2 ± 0.4 b
glutamic acid	132.9 ± 5.0 ab	119.1 ± 3.4 a	121.8 ± 9.0 ab	119.1 ± 9.4 a	117.0 ± 7.9 a	118.3 ± 4.5 a	136.8 ± 0.7 b
glutamine	175.6 ± 6.5 d	158.7 ± 5.3 c	156.3 ± 0.9 bc	154.0 ± 2.1 bc	191.5 ± 9.9 e	145.2 ± 0.7 b	130.2 ± 1.5 a
glycine	2.1 ± 0.1 ab	1.8 ± 0.1 ab	1.8 ± 0.1 ab	2.0 ± 0.2 ab	2.2 ± 0.6 b	1.9 ± 0.1 ab	1.6 ± 0.1 a
histidine	5.1 ± 0.6 a	6.1 ± 0.3 bc	6.0 ± 0.4 bc	5.1 ± 0.2 a	6.6 ± 0.1 c	5.3 ± 0.3 ab	5.1 ± 0.1 a
isoleucine	25.3 ± 0.7 a	25.3 ± 0.7 a	25.7 ± 1.2 a	25.5 ± 1.4 a	29.1 ± 0.9 b	23.4 ± 1.6 a	24.9 ± 0.9 a
leucine	22.9 ± 1.9 a	20.2 ± 0.9 a	20.9 ± 1.0 a	21.6 ± 0.4 a	23.2 ± 2.1 a	21.7 ± 0.6 a	20.6 ± 0.7 a
lysine	11.5 ± 1.3 ab	15.9 ± 1.1 c	15.9 ± 0.5 c	12.7 ± 0.4 abc	16.1 ± 2.1 c	14.5 ± 2.3 bc	11.1 ± 0.9 a
phenylalanine	25.2 ± 1.7 a	23.7 ± 0.6 a	25.3 ± 2.3 a	24.2 ± 0.6 a	30.0 ± 0.9 b	22.3 ± 0.5 a	22.8 ± 2.1 a
proline	20.4 ± 0.3 c	21.8 ± 1.6 b	21.5 ± 0.9 bc	20.4 ± 0.3 c	25.5 ± 1.4 bc	21.3 ± 1.6 bc	20.8 ± 0.2 a
serine	107.9 ± 3.1 c	99.2 ± 1.0 b	104.2 ± 0.9 bc	104.9 ± 0.9 c	103.7 ± 3.3 bc	103.4 ± 1.9 bc	87.1 ± 0.6 a
theanine	488.5 ± 9.9 d	480.1 ± 6.4 d	458.4 ± 7.2 c	438.4 ± 1.7 b	477.7 ± 2.2 d	442.0 ± 14.3 bc	399.4 ± 2.2 a
trptophan	24.1 ± 0.7 ab	23.2 ± 0.6 ab	24.1 ± 0.2 ab	26.7 ± 4.4 b	25.9 ± 0.9 ab	22.9 ± 0.9 ab	22.0 ± 0.7 a
tyrosine	44.9 ± 2.2 cd	36.7 ± 2.7 a	43.3 ± 1.8 bcd	40.4 ± 1.2 ab	46.9 ± 0.9 d	38.1 ± 2.3 a	40.8 ± 0.4 abc
valine	38.9 ± 1.3 c	33.1 ± 0.3 a	35.9 ± 0.9 b	35.9 ± 0.8 b	40.1 ± 0.4 c	34.6 ± 0.8 ab	34.9 ± 1.9 ab
total	1503 ± 39 d	1438 ± 27 c	1415 ± 10 bc	1383 ± 12 bc	1515 ± 12 d	1368 ± 37 b	1287 ± 5 a

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

the total organic acids present. Different organic acids (acetic, ascorbic, citric, glucuronic, isocitric, lactic, malic, oxalic, quinic, salicylic, shikimic, succinic, and tartaric) have been reported in different types of black teas at varying concentrations.[20,50−53] Organic acids have generally been reported to be responsible for sour, tart, acidic, and characteristic fruity taste of many foods.[54−57] Tannic acid, which was the predominant organic acid in black tea, has a characteristic astringency taste.[35,36] Moreover, theaflavins and thearubigins together with tannic acid could be responsible for the astringency taste in black tea.

Free Amino Acids. Table 7 shows the free amino acids present in seven grades of black tea. Black tea grades contained large amounts of theanine (399.4−488.5 mg/100 g), glutamine (130.2−191.5 mg/100 g), glutamic acid (117.0−136.8 mg/100 g), alanine (103.5−131.2 mg/100 g), and serine (87.1−107.9 mg/100 g). These five free amino acids constituted average 67.4% of the total free amino acids. In general, low-quality grades of black tea (grades 4−7) had lower free amino acids content than that of their high-quality counterparts (grades 1−3), except grade 5. Significant variations ($p < 0.05$) existed among seven grades of black tea (Table 7), with some exceptions. Despite the fact that all 18 free amino acids have a characteristic taste to the flavor of the black tea, theanine has a special attention due to its beneficial health benefits[58−60] as well as umami taste. It is the predominant amino acid in green tea leaves, giving tea its characteristic umami or "fifth taste" (besides the four traditional tastes: sweet, salty, acid, and bitter).[38,39] In the sensory part of this study, umami taste was not detected in any tea grade. The sweet and umami tastes result from the presence of theanine and glutamate in green tea.[37] Wang et al.[61] reported that theanine levels as 88.4−136.9 mg/100 g for black tea and 215−402 mg/100 g for green tea. On the other hand, Keenan et al.[62] found that the amount of theanine present in black tea samples was significantly greater than in either black specialty, green, or white tea varieties having the levels up to 910 mg/100 g. However, the theanine content varies in accordance with a variety of factors, including growing location, method of cultivation, tea grade, variety, and time of harvest, among others.

The presence and composition of taste-active components (sugars, organic acids, and free amino acids) of black tea may be affected by various factors such as variety, growing condition, maturity, season, geographic origin, fertilization, soil type, storage conditions, amount of sunlight received, and time of harvest, among others

The present work suggests that certain flavor attributes correlated well with taste- and aroma-active compounds. Some variations ($p < 0.05$), albeit to different extents, were observed among volatile compounds, sugars, organic acids, and free amino acids in seven grades of black tea. However, high-and low-quality black teas should not be distinguished solely on the basis of their DSA and taste- and aroma-active compounds.

AUTHOR INFORMATION

Corresponding Author

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

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REFERENCES

(1) FAO. *FAOSTAT-Tea Production 2009*; FAO: Rome, Italy, 2009.

(2) Wang, L.; Park, S.; Chung, J.; Baik, J.; Park, S. The compounds contributing to the greenness of green tea. *J. Food Sci.* **2004**, *69*, S301−S305.

(3) Rawat, R.; Gulati, A.; Kiran Babu, G. D.; Acharya, R.; Kaul, V. K.; Singh, B. Characterization of volatile components of Kangra orthodox black tea by gas chromatography mass spectrometry. *Food Chem.* **2007**, *105*, 229−235.

(4) Borse, B. B.; Rao, L. J. M.; Nagalakshmi, S.; Krishnamurthy, N. Fingerprint of black teas from India: identification of the regio-specific characteristics. *Food Chem.* **2002**, *79*, 419−424.
(5) Bhattacharyya, N.; Bandyopadhyay, R.; Bhuyan, M.; Tudu, B.; Ghosh, D.; Jana, A. Electronic nose for black tea classification and correlation of measurements with "tea taster" marks. *IEEE Trans. Instrum. Meas.* **2008**, *57*, 1313−1321.
(6) Bondarovich, H.; Giammarino, A. S.; Renner, J. A.; Shephard, F. W.; Shingler, A. J.; Gianturco, M. A. Some aspects of the chemistry of tea. A contribution to the knowledge of the volatile constituents. *J. Agric. Food Chem.* **1967**, *15*, 36−47.
(7) Renold, W.; Näf-Müller, R.; Keller, U.; Willhalm, B.; Ohloff, G. An investigation of the tea aroma part I. New volatile black tea constituents. *Helv. Chim. Acta* **1974**, *57*, 1301−1308.
(8) Vitzthum, O. G.; Werkhoff, P.; Hubert, P. New volatile constituents of black tea aroma. *J. Agric. Food Chem.* **1975**, *23*, 999−1003.
(9) Mick, W.; Goetz, E.; Schreier, P. Volatile acids of black tea aroma. *Lebensm. Wiss. Technol.* **1984**, *17*, 104−106.
(10) Mick, W.; Schreier, P. Additional volatiles of black tea aroma. *J. Agric. Food Chem.* **1984**, *32*, 924−929.
(11) Shimoda, M.; Shigematsu, H.; Shiratsuchi, H.; Osajima, Y. Comparison of the odor concentrates by SDE and adsorptive column method from green tea infusion. *J. Agric. Food Chem.* **1995**, *43*, 1616−1620.
(12) Kumazawa, K.; Masuda, H. Identification of potent odorants in different green tea varieties using flavor dilution technique. *J. Agric. Food Chem.* **2002**, *50*, 5660−5663.
(13) Schuh, C.; Shieberle, P. Characterization of the key aroma compounds in the beverage prepared from Darjeeling black tea: Quantitative differences between tea leaves and infusion. *J. Agric. Food Chem.* **2006**, *54*, 916−924.
(14) Wang, L. F.; Lee, Y. J.; Chung, J. O.; Baik, J. H.; So, S.; Park, S. K. Discrimination of teas with different degrees of fermentation by SPME-GC analysis of the characteristic volatile flavour compounds. *Food Chem.* **2008**, *109*, 196−206.
(15) Wang, K.; Liu, F.; Liu, Z.; Huang, J.; Xu, Z.; Li, Y.; Chen, J.; Gong, Y.; Yang, X. Comparison of catechins and volatile compounds among different types of tea using high performance liquid chromatograph and gas chromatograph mass spectrometer. *Int. J. Food Sci. Technol.* **2011**, *46*, 1406−1412.
(16) Robertson, A. The chemistry and biochemistry of black tea production: The non-volatiles. In *Tea: Cultivation to Consumption*; Wilson, K. C., Clifford, M. N., Eds.; Chapman and Hall: London, 1992; pp 553−601.
(17) McDowell, I.; Taylor, S.; Gay, C. The phenolic pigment composition of black tea liquors. Part I. Predicting quality. *J. Sci. Food Agric.* **1995**, *69*, 467−474.
(18) Shahidi, F.; Naczk, M. *Phenolics in Foods and Nutraceuticals*; CRC Press: Boca Raton, FL, 2004.
(19) Varnam, A. H.; Sutherland, V. J. *Beverages: Technology, Chemistry and Microbiology*; Springer: Heidelberg, Germany, 1994.
(20) Harbowy, M. E.; Balentine, D. A.; Davies, A. P.; Cai, Y. Tea chemistry. *Crit. Rev. Plant Chem.* **1997**, *16*, 415−480.
(21) ISO 6564. *Sensory Analysis-Methodology-Flavour Profile Methods*; International Organizations for Standardization: Geneva, Switzerland, 1985.
(22) Alasalvar, C.; Grigor, J. M.; Quantick, P. C. Method for the static headspace análisis of carrot volatiles. *Food Chem.* **1999**, *65*, 391−397.
(23) Alasalvar, C.; Shahidi, F.; Cadwallader, K. R. Comparison of natural and roasted Turkish hazelnut (*Corylus avellana* L.) volatiles and flavor by DHA/GC/MS and descriptive sensory analysis. *J. Agric. Food Chem.* **2003**, *51*, 5067−5072.
(24) Alasalvar, C.; Taylor, K. D. A.; Shahidi, F. Comparison of volatiles of cultured and wild sea bream (*Sparus aurata*) during storage in ice by dynamic headspace analysis/gas chromatography-mass spectrometry. *J. Agric. Food Chem.* **2005**, *53*, 2616−2622.
(25) van den Dool, H.; Kratz, P. D. A generalization of the retention index system including linear temperature programmed gas liquid chromatography. *J. Chromatogr.* **1963**, *11*, 463−471.
(26) Serpen, A.; Gökmen, V. Evaluation of the Maillard reaction in potato crisps by acrylamide, antioxidant capacity and color. *J. Food Compos. Anal.* **2009**, *22*, 589−595.
(27) Gökmen, V.; Serpen, A.; Mogol, B. A. Rapid determination of amino acids in foods by hydrophilic interaction liquid chromatography coupled to high resolution mass spectrometry. *Anal. Bioanal. Chem.* **2012**, DOI: 10.1007/s00216-012-5779-z.
(28) Kovács, Z.; Dalmadi, I.; Lukács, L.; Sipos, L.; Szántai-Kőhegyi, K.; Kókai, Z.; Fekete, A. Geographical origin identification of pure Sri Lanka tea infusions with electronic nose, electronic tongue and sensory profile analysis. *J. Chemom.* **2010**, *24*, 121−130.
(29) Obanda, M.; Owuor, P. O.; Mang'oka, R. Changes in the chemical and sensory quality parameters of black tea due to variations of fermentation time and temperature. *Food Chem.* **2001**, *75*, 395−404.
(30) He, W.; Hu, X.; Zhao, L.; Liao, X.; Zhang, Y.; Zhang, M.; Wu, J. Evaluation of Chinese tea by the electronic tongue: Correlation with sensory properties and classification according to geographical origin and grade level. *Food Res. Int.* **2009**, *42*, 1462−1467.
(31) Liang, Y.; Lu, J.; Zhang, L.; Wu, S.; Wu, Y. Estimation of black tea quality by analysis of chemical composition and colour difference of tea infusions. *Food Chem.* **2003**, *80*, 283−290.
(32) Kumazawa, K.; Masuda, H. Change in the flavour of black tea drink during heat processing. *J. Agric. Food Chem.* **2001**, *49*, 3304−3309.
(33) Ahmed, S.; Unachukwu, U.; Stepp, J. R.; Peters, C. M.; Long, C.; Kennelly, E. Pu-erh tea tasting in Yunnan, China: Correlation of drinkers' perceptions to phytochemistry. *J. Ethnopharmacol.* **2010**, *132*, 176−185.
(34) Alexander, R. J. *Sweeteners: Nutritive*; Eagan Press: St. Paul, MN, 1998.
(35) Critchley, H. D.; Rolls, E. T. Responses of primate taste cortex neurons to the astringent tastant tannic acid. *Chem. Senses* **1996**, *21*, 135−145.
(36) Rolls, E. T. Taste and olfactometry processing in the brain and its relation to the control of eating. *Crit. Rev. Neurobiol.* **1997**, *11*, 263−287.
(37) Muramatsu, K. *Science of Tea*; Asakura Publishing Co., Ltd.: Tokyo, Japan, 1991 (in Japanese).
(38) Suzuki, H.; Izuka, S.; Miyakawa, N.; Kumagai, H. Enzymatic production of theanine, an 'umami' component of tea, from glutamine and ethylamine with bacterial γ-glutamyltranspeptidase. *Enzyme Microb. Technol.* **2002**, *31*, 884−889.
(39) Wan, X.; Zhang, Z.; Li, D. Chemistry and biological properties of theanine. In *Tea and Tea Products: Chemistry and Health-Promoting Properties*; Ho, C.-T., Lin, J.-K., Shahidi, F., Eds.; CRC Press, Taylor & Francis Group: Boca Raton, FL, 2009; pp 255−274.
(40) Chung, H. Y.; Cadwallader, K. R. Volatile components in blue crab (*Callinectes sapidus*) meat and processing by-products. *J. Food Sci.* **1993**, *58*, 1203−1207, 1211.
(41) Ravichandran, R.; Parthiban, R. Lipid occurrence, distribution and degredation to flavour volatiles during tea processing. *Food Chem.* **2000**, *68*, 7−13.
(42) Heath, H. B.; Reineccius, G. Flavor and its study. In *Flavor Chemistry and Technology*; Heath, H., Reineccius, G., Eds.; AVI Publishing: Westport, CT, 1986; pp 71−111.
(43) Tanchotikul, U.; Hsieh, T. C.-Y. Analysis of volatile flavor components in steamed rangia clam by dynamic headspace sampling and simultaneous distillation and extraction. *J. Food Sci* **1991**, *56*, 327−331.
(44) Josephson, D. B.; Lindsay, R. C. Enzymic generation of volatile aroma compounds from fresh fish. In *Biogeneration of Aroma*; Parliament, T. H., Croteau, R., Eds.; American Chemical Society: Washington, DC, 1986; pp 201−219.
(45) Chung, H. Y.; Cadwallader, K. R. Aroma extract dilution analysis of blue crab claw meat volatiles. *J. Agric. Food Chem.* **1994**, *42*, 2867−2870.

(46) Pan, B. S.; Kou, J.-M. Flavour of shellfish and kamaboko flavourants. In *Seafoods Chemistry, Processing Technology and Quality*; Shahidi, F., Botta, J. R., Eds.; Blackie Academic & Professional: Glasgow, United Kingdom, 1994; pp 85−114.
(47) Shibamoto, T.; Akiyama, T.; Sakaguchi, M.; Enomoto, Y.; Masuda, H. A study of pyrazine formation. *J. Agric. Food Chem.* **1979**, *27*, 1027−1031.
(48) Wong, J. M.; Bernhard, R. A. Effect of nitrogen source on pyrazine formation. *J. Agric. Food Chem.* **1988**, *36*, 123−129.
(49) Maga, J. A. Furans in food. *CRC. Crit. Rev. Food Sci. Nutr.* **1979**, *11*, 355−400.
(50) Ding, M.-Y.; Chen, P.-R.; Luo, G.-A. Simultaneous determination of organic acids and inorganic anions in tea by ion chromatography. *J. Chromatogr.* **1997**, *764*, 341−435.
(51) Souci, S. W., Fachmann, W., Kraut, H. *Food Composition and Nutrition Tables*, 7th revised and completed ed.; CRC Press, Taylor & Francis Group: Boca Raton, FL, 2008.
(52) Alcázar, A.; Fernández-Cáceres, P. L.; Martín, M. J.; Pablos, F.; González, A. G. Ion chromatographic determination of some organic acids, choloride and phosphate in coffee and tea. *Talanta* **2003**, *61*, 95−101.
(53) Jayabalan, R.; Marimuthu, S.; Swaminathan, K. Changes in content of organic acids and tea polyphenols during kombucha tea fermentation. *Food Chem.* **2007**, *102*, 392−398.
(54) Johnston, F. B.; Hammill, M. M. The nonvolatile organic acids of some fresh fruits and vegetables. *Can. Inst. Food Technol. J.* **1968**, *1*, 3−5.
(55) Ulrich, R. Organic acids. In *The Biochemistry of Fruits and Their Products*; Hulme, A. C., Ed.; Academic Press: New York, NY, 1970; Vol. *1*, pp 89−118.
(56) Jadhav, S. J.; Andrew, W. T. Effects of cultivars and fertilizers on nonvolatile organic acids in potato tubers. *Can. Inst. Food Sci. Technol. J.* **1977**, *10*, 13.
(57) Siebert, K. J. Modeling the flavor thresholds of organic acids in beer as a function of their molecular properties. *Food Qual. Pref.* **1999**, *10*, 129−137.
(58) Huber, L. G. Green tea catechins and L-theanine in integrative cancer care: A review of the research. *Altern. Complement. Ther.* **2003**, *9*, 294−298.
(59) Bryan, J. Psychological effects of dietary components of tea: caffeine and L-theanine. *Nutr. Rev.* **2007**, *66*, 82−90.
(60) Nobre, A. C.; Rao, A.; Owen, G. N. L-theanine, a natural constituent in tea, and its effect on mental state. *Asia Pac. J. Clin. Nutr.* **2008**, *17*, 167−168.
(61) Wang, L.; Xu, R.; Li, W.; Sun, Y.; Tu, Y.; Zeng, X. Analysis of free amino acids in Chinese teas and flower of tea plant by high performance liquid chromatography combined with solid-phase extraction. *Food Chem.* **2010**, *123*, 1259−1266.
(62) Keenan, E. K.; Finnie, M. D. A.; Jones, P. S.; Rogers, P. J.; Priestley, C. M. How much theanine in a cup of tea? Effects of tea type and method of preparation. *Food Chem.* **2011**, *125*, 588−594.
(63) ISO 3103. *Tea-Preparation of Liquor for Use in Sensory Tests*; International Organizations for Standardization: Geneva, Switzerland, 1980.