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Compositional, Nutritional, and Functional Characteristics of Instant Teas Produced from Low- and High-Quality Black Teas

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ABSTRACT: Two types of instant teas produced from low- and high-quality black teas were examined for their proximate composition, dietary fiber, minerals, water-soluble vitamins, total phenolic content, various antioxidant assays, phenolics (flavanols, condensed phenolics, and phenolic acids), alkaloids, and carotenoids as well as taste-active compounds (sugars, organic acids, and free amino acids). Some variations, albeit to different extents, were observed ($p < 0.05$) among these parameters between instant teas produced from low- and high-quality black teas. With respect to proximate composition, carbohydrate was the predominant component (56.68−59.84 g/100 g), followed by protein (19.31−19.86 g/100 g). Ash, moisture, and, to a lesser extent, dietary fiber and fat were also present in both instant teas. Thirteen minerals, four water-soluble vitamins, six flavanols, two alkaloids, three condensed phenolics, one phenolic acid, and one carotenoid were identified. Total phenolic content varied between 17.35 and 17.82 g of gallic acid equivalents (GAE)/100 g instant tea. With regard to antioxidant activities, three different assays such as oxygen radical absorbance capacity (ORAC), trolox equivalent antioxidant capacity (TEAC), and cupric ion reducing antioxidant capacity (CUPRAC) were measured. No significant differences ($p > 0.05$) in total phenolic, ORAC, TEAC, and CUPRAC contents between low- and high-quality instant teas were observed. With regard to taste-active compounds, 3 sugars, 5 organic acids, and 18 free amino acids were positively identified, of which fructose, tannic acid, and theanine predominated, respectively. The present work suggests that despite some differences, instant teas produced from low- and high-quality black teas should not be distinguished on the basis of their compositional, nutritional, and functional characteristics as well as taste-active compounds.

KEYWORDS: *instant tea, minerals, water-soluble vitamins, carotenoids, phenolics, antioxidant activity, taste-active compounds*

INTRODUCTION

Tea is one of the most pleasant and popular beverages in the world. Black tea consumed throughout the world is believed to be not only a popular beverage but also an antioxidative agent available in every day life, which may help to prevent a wide variety of diseases.[1,2] The world's tea production in 2011 was around 4,602,193 MT. China is the world's largest producer of tea, contributing 35.6% to the total global production, followed by India (21.0%), Kenya (8.2%), Sri Lanka (7.1%), Turkey (4.8%), Vietnam (4.5%), and Iran (3.5%). Other countries contribute 15.3% to the total global production. Turkey, as the fifth largest producer of tea, has a production of 221,600 MT.[3] Black tea accounts for ∼78% to the total tea production, mainly consumed in the Western countries.

Black tea is known to consist of considerable amounts of phytochemicals apart from some nutritional compounds present in limited quantities.[4−6] Considerable interest has developed in the past decade in unraveling the beneficial effects of black tea, particularly in polyphenolic components and antioxidant activity. Flavanols (catechins), theaflavins, and thearubigins are three important groups of polyphenols present in black tea.[4−8] The formation mechanisms of these compounds during tea processing and fermentation as well as their respective biological activities are of great importance and of scientific and commercial interest.

The common process of black tea production consists of four stages, namely, withering, rolling, fermentation, and firing. 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), low-quality tea (grades 4−7)].[9]

There are a number of products in the market, namely, blended tea, loose tea, tea bag, tea packet, instant tea, ready-to-drink tea, and flavored tea, among others.[10] Instant tea powder is the fully soluble solid tea product that has emerged as a new fast-growing commodity in every country. Three types of conventional methods exist to produce instant tea powder, namely, spray-, freeze-, and vacuum-dryings.[11] Spray-drying is a widely used and well-established technique to produce powder from liquid and semiliquid foods.[12] Small liquid droplets are quickly dried by inlet hot air during concurrent spray-drying.[13] Spray-drying is a short and controllable process, and products retain high quality properties such as color, flavor, and nutrients. Different drying aids or carrier materials, such as maltodextrin, modified starches, and arabic gum, have been

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used in the spray-drying process to provide stability and improve product recovery.[14] Several constituents in tea, such as volatile compounds and polyphenols, are thermally unstable and may degrade during the thermal extraction, which directly influences the flavor and color of the final product.[11] The epistructured catechins tend to be converted to their nonepistructured counterparts or be degraded due to oxidation at high temperatures.[14−16]

Spray-drying and agglomeration are widely used for the production of instant tea. Effects of drying temperatures and processing on compositional, nutritional, and functional characteristics of the final product need detail evaluation. The change of these parameters in instant tea is of great importance and of commercial interest, and no detailed data exist in the literature. Therefore, the objective of this study was to compare the compositional, nutritional, and functional characteristics of instant teas produced from low- and high-quality black teas.

■ MATERIALS AND METHODS

Samples. Low-quality (grades 4−7) and high-quality (grades 1−3) black teas were procured from the ÇAYKUR Tea Processing Plant (Rize, Turkey) 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 processed. Low-quality (grades 4−7) and high-quality (grades 1−3) black teas were mixed separately at the same amount prior to processing. All samples were processed 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.

Production of Instant Tea. *Extraction of Black Tea Samples.* A schematic of the process used to produce the instant black tea powder is presented in Figure 1. The extraction process was performed in a pilot-scale continuous extractor (Niro Atomizer, AC-27, Soeborg, Denmark). The operational conditions for the continuous extractor were as follows: water inlet temperature (80−85 °C), jacket temperature (80−85 °C), tea feed rate (12 kg/h), water feed rate (42 L/h), and slope of the extractor (3−5°). The extract was centrifuged (Westfelia, D-4740, North Rhine-Westphalia, Germany) at 17000*g* before feeding to the spray-dryer.

Spray-Drying. Spray-drying was performed in Minor Spray Dryer (Niro Atomizer), which has a centrifugal atomizer (inside diameter = 120 mm) and operates in a concurrent manner. The operational conditions for the spray-dryer were as follows: inlet air temperature (170−180 °C) and outlet air temperature (120−125 °C). The feed rate was set as 7.6 L/h.

Agglomeration. A fluidized bed reactor (GLATT, Procell Lab System, Weimar, Germany) was used for agglomeration of instant black tea powder, under the following conditions: air flow rate (105−80 m^3/h), air temperature (85 °C), exhaust air temperature (45−50 °C), product temperature (50−55 °C), spraying pressure (2.5 bar), and pump position (3−8).

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.[17] Percentage crude protein was estimated by multiplying the total nitrogen content by a factor of 6.25.[17] 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).[17] 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.

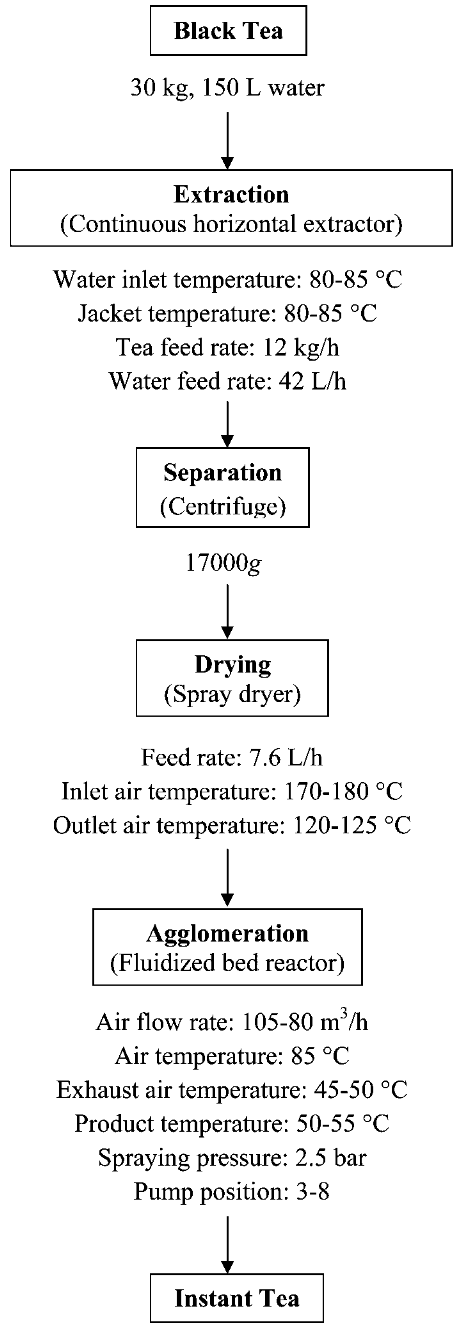

Figure 1. Flowchart for production of instant tea.

Determination of Minerals. Minerals were analyzed according to AOAC method 999.10.[17] They were determined using an inductively coupled plasma−mass spectrometer (ICP-MS) (Elan DRCE, Perkin-Elmer, Norwalk, CT, USA) as detailed by Serpen et al.[6] Values were expressed as milligrams per 100 g of instant tea.

Determination of Water-Soluble Vitamins. Water-soluble vitamins 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[18] and detailed by Serpen et al.[6] Values were expressed as milligrams per 100 g of instant tea.

Determination of Carotenoids. Carotenoids were extracted and analyzed according to the method described by Gökmen et al.[19] and Serpen et al.[6] Chromatographic analyses were performed on an Agilent 1200 high-performance liquid chromatography (HPLC) system consisting of a diode array detector (DAD), quaternary pump, autosampler, and column oven (Agilent Technologies, Waldbronn, Germany). An Agilent Eclipse XDB-C8 column (150 mm × 4.6 mm, 5 μm particles, Agilent Technologies) was used to separate carotenoids. The quantitation of carotenoids was based on calibration curves built for each of the compounds identified in instant tea samples. Data were expressed as milligrams of carotenoid per 100 g of instant tea.

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.[20] 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 instant tea.

Determination of Oxygen Radical Absorbance Capacity (ORAC). The antioxidant activity was determined according to the ORAC assay as described by Wu et al.[21] 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 instant tea.

Determination of Trolox Equivalent Antioxidant Capacity (TEAC). The antioxidant activity was measured according to the TEAC assay as detailed by Dubeau et al.[22] Samples were extracted by 70% methanol as described by ISO 14502-2:2005,[20] 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 instant tea.

Determination of Cupric Ion Reducing Antioxidant Capacity (CUPRAC). The method described by Apak et al.[23] was used to assess the CUPRAC extract and its fraction. Samples were extracted by 70% methanol as described by ISO 14502-2:2005,[20] and the analysis was performed on the diluted samples as detailed by Apak et al.,[23] 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 instant tea.

Determination of Phenolic and Alkaloid Compounds. Phenolic compounds were extracted and analyzed according to the HPLC method of Dou et al.[24] as outlined in detail by Serpen et al.,[6] with some minor modifications. Chromatographic analyses were performed on a Shimadzu HPLC system (LC-20AD pump, SPD-M20A DAD detector, SIL-20A HT autosampler, CTO-2OAC column oven, DGU-20A$_5$ degasser, and CMB-20A communications bus module, Shimadzu Corp., Kyoto, Japan). An Atlantis dC18 column (250 mm × 4.6 mm, 5 μm particles, Waters Corp.) was used to separate flavanols, alkaloids, and phenolic acids in instant 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). The quantitations of flavanols, alkaloids, and phenolic acids were based on calibration curves built for each of the compounds identified in instant teas. Data were expressed as milligrams per 100 g of instant tea.

Determination of Theaflavin. Theaflavin was extracted according to the methods of Neilson et al.[25] and Mulder et al.,[26] as detailed by Serpen et al.,[6] with some minor modifications. The equipment consisted of an ultrafast liquid chromatography (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). A Luna Phenyl Hexyl column (250 × 4.6 mm, 5 μm particles, Phenomenex, Cheshire, UK) was used at 30 °C for the chromatographic separation of theaflavin. 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. The gradient profile was programmed as follows: 0−6 min, linear gradient from 20 to 80%; 6−11 min, isocratic elution 80% B; 11−11.5 min, linear gradient from 80 to 20%; and 11.5−13 min, isocratic elution 20% B. Theaflavin was quantified on the basis of peak area and comparison with a calibration curve obtained with the corresponding standard. Data were expressed as milligrams of theaflavin per 100 g of instant tea.

Determination of Thearubigins. *Preparation of Thearubigin Standard.* Thearubigins were extracted according to the caffeine precipitation method of Kuhnert et al.[27] as detailed by Serpen et al.,[6] with some minor modifications.

Sample Extraction. Thearubigins in instant tea samples were extracted according to a procedure described by Tanaka et al.[28] and Serpen et al.[6] A Spe-ed C18/18 (Applied Separations, Allentown, PA, USA) cartridge was used for the filtration of the extracts, and chromatographic analyses were performed on a Shimadzu HPLC system (LC-20AD pump, SPD-M20A DAD detector, SIL-20A HT autosampler, CTO-2OAC column oven, DGU-20A$_5$ degasser, and CMB-20A communications bus module, Shimadzu Corp.) with a Kromosil C-18 column (150 mm × 4.6 mm, 5 μm particles, Teknokroma, Barcelona, Spain) at 35 °C. A gradient of mobile phases A (acetonitrile) and B (50 mM phosphoric acid) was used, at a flow rate of 0.8 mL/min, for the chromatographic separation. The gradient profile was programmed as follows: 0−18 min, linear gradient from 10 to 23% A; 18−19 min, linear gradient from 23 to 90% A; 19−29 min, isocratic elution 90% A; 29−30 min, linear gradient from 90 to 20% A; and 30−35 min isocratic elution 20% A. 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.[27] Data were expressed as milligrams of thearubigins per 100 g of instant tea.

Determination of Sugars. Sugar levels were measured according to the HPLC method of Alasalvar et al.,[9] with some modifications. Chromatographic analyses were performed on an Agilent 1200 HPLC system consisting of a refractive index (RI) detector, quaternary pump, autosampler, and column oven (Agilent Technologies) with Shodex Rspak KC-811 column (300 mm × 7.8 mm, 6 μm particles, Shoko Corp., Tokyo, Japan). Identified sugars were quantified on the basis of peak areas and comparison with a calibration curve obtained with the corresponding standards. Data were expressed as grams of sugar per 100 g of instant tea.

Determination of Organic Acids. Organic acids were extracted according to the HPLC method of Alasalvar et al.,[9] with some modifications. Chromatographic analyses were performed on an Agilent 1200 HPLC system consisting of a DAD, quaternary pump, autosampler, and column oven (Agilent Technologies) with a Shodex Rspak KC-811 column (300 mm × 7.8 mm, 6 μm particles, Shoko Corp.). The quantitation of organic acids was based on calibration curves built for each of the compounds identified in instant tea samples. Data were expressed as grams of organic per 100 g of instant tea.

Determination of Free Amino Acids. Free amino acids were extracted according to the method described by Alasalvar et al.[9] and Kocadağlı et al.[29] Free amino acid analysis was performed using hydrophilic interaction liquid chromatography with mass spectrometric detection. An Agilent 1200 HPLC system coupled to an Agilent 6460 triple-quadrupole mass spectrometer was used. Concentration of free amino acids was calculated by means of external calibration curves built for individual amino acids in a range between 0.1 and 2.0 μg/L. Data were expressed as milligrams of free amino acid per 100 g of instant tea.

Statistical Analysis. Results were expressed as the mean ± the 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 SPSS 18.0 version (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Proximate Composition and Dietary Fiber. Table 1 shows the proximate composition and dietary fiber content of low- and high-quality instant teas. Carbohydrate was the predominant component (56.68–59.84 g/100 g), followed by protein (19.31–19.86 g/100 g). These values were comparable with those reported by the USDA for instant tea.[30] Ash, moisture, and, to a lesser extent, dietary fiber and fat were also present in both instant teas. Significant variations between low- and high-quality instant teas were observed ($p < 0.05$), except for insoluble dietary fiber. The dietary fiber content of low-quality instant tea was 2.4-fold higher than that of its high-quality counterpart. It is interesting to note that dietary fiber contents of instant teas were somewhat lower (1.28–3.05 g/100 g) than those reported by the USDA (8.5 g/100 g).[30]

Table 1. Compositional and Nutritional Characteristics of Instant Teas (Values per 100 g of Instant Tea)[a]

	unit	instant tea produced from: low quality	instant tea produced from: high quality
proximate composition			
moisture	g	8.54 ± 0.02a	9.26 ± 0.01b
ash	g	10.77 ± 0.02a	9.57 ± 0.03b
protein	g	19.86 ± 0.04a	19.31 ± 0.07b
dietary fiber	g	3.05 ± 0.11a	1.28 ± 0.13b
soluble	g	1.81 ± 0.22a	0.19 ± 0.07b
insoluble	g	1.24 ± 0.12a	1.09 ± 0.07a
fat	g	1.10 ± 0.03a	0.74 ± 0.03b
carbohydrate	g	56.68 ± 0.08a	59.84 ± 0.10b
minerals			
calcium	mg	73.0 ± 3.5a	103 ± 4b
chromium	mg	0.17 ± 0.01a	0.16 ± 0.01a
copper	mg	0.47 ± 0.01a	0.99 ± 0.10b
cobalt	mg	0.07 ± 0.00a	0.06 ± 0.00b
iron	mg	3.22 ± 0.27a	2.46 ± 0.11b
magnesium	mg	280 ± 11a	179 ± 3b
manganese	mg	151 ± 2a	166 ± 4b
molybdenum	mg	0.42 ± 0.02a	0.21 ± 0.00b
phosphorus	mg	431 ± 4a	376 ± 12b
potassium	mg	4625 ± 101a	3928 ± 91b
selenium	mg	tr	tr
sodium	mg	5.41 ± 0.45a	4.41 ± 0.27a
zinc	mg	2.85 ± 0.08a	1.78 ± 0.13b
vitamins			
thiamin	mg	0.09 ± 0.01a	0.13 ± 0.03a
niacin	mg	20.64 ± 0.56a	20.85 ± 0.46a
pantothenic acid	mg	62.38 ± 0.81a	59.08 ± 0.74b
pyridoxine	mg	0.46 ± 0.01a	0.37 ± 0.01b
carotenoid			
lutein	mg	1.24 ± 0.10a	0.51 ± 0.06b

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

Minerals. Thirteen minerals (calcium, chromium, copper, cobalt, iron, magnesium, manganese, molybdenum, phosphorus, potassium, selenium, sodium, and zinc) were studied for the first time in low- and high-quality instant teas, and the results are given in Table 1. Among the minerals, potassium was most abundant (3928–4625 mg/100 g), followed by phosphorus (376–431 mg/100 g), magnesium (179–280 mg/100 g), manganese (151–166 mg/100 g), and calcium (73–103 mg/100 g). No significant differences ($p > 0.05$) in chromium, selenium, and sodium contents between low- and high-quality instant teas were observed.

Seven minerals such as calcium, iron, magnesium, phosphorus, potassium, sodium, and zinc were reported in instant tea by the USDA,[30] and values are within the range of the present study except for sodium, which was ~15-fold higher. The 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.[6]

Water-Soluble Vitamins. Among the four water-soluble vitamins (thiamin, niacin, pantothenic acid, and pyridoxine) detected in instant teas produced from low- and high-quality black teas, pantothenic acid was most abundant, with its concentration up to 59.08–62.38 mg/100 g, followed by niacin (20.64–20.85 mg/100 g), and, to a lesser extent, pyridoxine (0.37–0.46 mg/100 g) and thiamin (0.09–0.13 mg/100 g) (Table 1). Significant differences ($p < 0.05$) in pantothenic acid and pyridoxine existed between low- and high-quality black teas. Other water-soluble vitamins were not detected in any of the instant black teas. Serpen et al.[6] studied the nutritional and functional characteristics of seven grades of black tea produced in Turkey and found the same number of water-soluble vitamins.

The USDA[30] reported four water-soluble vitamins in instant tea (riboflavin, niacin, pantothenic acid, and folate), of which niacin was most abundant (10.8 mg/100 g), followed by riboflavin (0.985 mg/100 g), pyridoxine (0.356 mg/100 g), and folate (0.103 mg/100 g). Niacin content was 2-fold higher in the present study than the value reported by the USDA.[30] The observed differences could be due to variety or processing.

Carotenoid. Table 1 shows the levels of carotenoids in low- and high-quality instant teas. Although both black tea grades contained remarkable amounts of chlorophylls (chlorophyll *a* and chlorophyll *b*) and carotenoids (lutein and β-carotene),[6] instant teas obtained from these teas were found to contain only a trace amount of lutein (0.51–1.24 mg/100 g). This could be due to the processing at high temperature (170–180 °C) using a spray-dryer. Instant tea produced from the low-quality black tea contained significantly higher ($p < 0.05$) lutein as compared to its high-quality counterpart. It is a fact that transfer of chlorophylls and carotenoids into the infusion is not favorable due to their limited solubility in water.

Total Phenolics and Antioxidant Activities. Total phenolic content and antioxidant activities using three different assays (ORAC, TEAC, and CUPRAC) were measured (Table 2). Total phenolic contents varied between 17.82 g of GAE/100 g in low-grade instant tea and 17.35 GAE/100 g in high-grade instant tea. No significant differences ($p > 0.05$) in total phenolic, ORAC, TEAC, and CUPRAC contents between low- and high-quality instant teas were observed. Instant tea has been reported to have high polyphenol content, and the corresponding value was 19.63%.[10]

Phenolics. Six major flavanols (catechin, epicatechin, epicatechin gallate, epigallocatechin, epigallocatechin gallate, and gallocatechin), three condensed phenolics (theaflavin, thearubigins, and theaflavin 3,3′-digallate), and one phenolic acid (gallic acid) were identified in low- and high-quality instant teas (Table 3). As expected, concentrations of phenolics detected in instant teas were much higher than those reported in seven grades of black tea.[6] This was due to the concentration of instant tea.

Table 2. Total Phenolic Content (Grams of GAE per 100 g) and Antioxidant Activities (Micromoles of TE per Gram) of Instant Teas[a]

	instant tea produced from	
	low quality	high quality
total phenolics	17.82 ± 0.68a	17.35 ± 0.60a
ORAC	1603 ± 311a	1968 ± 9a
TEAC	1455 ± 184a	1442 ± 140a
CUPRAC	2720 ± 126a	2829 ± 93a

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

Table 3. Phenolic and Alkaloid Contents of Instant Teas (Milligrams per 100 g)[a]

	instant tea produced from	
	low quality	high quality
flavanols		
catechin	288 ± 3a	316 ± 3b
epicatechin	889 ± 7a	524 ± 8b
epicatechin gallate	428 ± 7a	315 ± 5b
epigallocatechin	3460 ± 26a	2004 ± 13b
epigallocatechin gallate	176 ± 3a	173 ± 3a
gallocatechin	506 ± 2a	397 ± 3b
condensed phenolics		
theaflavin	56 ± 5a	124 ± 10b
theaflavin 3,3′-digallate	27 ± 1a	24 ± 0a
thearubigins	10161 ± 1608a	12701 ± 763a
phenolic acids		
gallic acid	751 ± 6a	697 ± 5b
alkaloids		
caffeine	4398 ± 34a	3964 ± 26b
theabromine	44 ± 1a	37 ± 2b

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

Instant tea produced from the low-quality black tea had a tendency to have higher flavanols, condensed phenolics, and gallic acid than instant tea produced from the high-quality black tea. Some variations ($p < 0.05$), except epigallocatechin gallate, theaflavin 3,3′-digallate, and thearubigins, in phenolics were noted between the instant teas. The variations of phenolic constituents could be attributed to the varying leaf quality and processing conditions.

Epigallocatechin and thearubigins were most abundant, representing 54–60 and 98–99%, to the total flavanols and condensed phenolics, respectively. Gallic acid was the only phenolic acid detected in instant teas.

Hakim et al.[31] analyzed 40 tea samples (representing the most typical preparation techniques of hot, iced, and sun tea) using HPLC for total flavonoids, catechins, theaflavin, thearubigins, caffeine, and gallic acid. In black tea, the highest concentrations of flavonoids were found in brewed hot tea (ranging from 541 to 692 μg/mL), whereas the lowest concentrations were for instant tea preparations (ranging from 91 to 100 μg/mL). They concluded that tea concentration, brewing time, and beverage temperature had major influences on flavonoid concentrations. Various phenolics have also been reported in instant teas at various concentrations.[10,11,32]

Flavanols detected in the present study were also reported in various black teas, albeit to different extents.[33−35] Black tea contains different extents and variety of condensed phenolic compounds owing to the polymerization reactions of monomeric phenolic substances.[36] Theaflavin levels have been reported to vary between 40 and 910 mg/100 g for black tea samples extracted with different solvents.[6,25,33,37] The thearubigins, which are the polymeric form of oxidized flavanols, are the dominant phenolics of black tea.[7] Balentine[33] reported that black tea leaves contained 5950 mg/100 g thearubigins (dry weight). Recently, Serpen et al.[6] found that thearubigin levels in seven grades of black tea were in the range of 5920–6830 mg/100 g. The reported values for thearubigins were 2-fold lower than in the present study.

The content of gallic acid in this study was almost 7-fold higher than those reported by Serpen et al.[6] in seven grades of black tea. The concentration and composition of phenolic acids in tea products depend not only on differences in processing but also tea plant cultivars and agricultural conditions.[7] Gallic acid has been reported in instant black tea at varying concentrations.[31,32]

Alkaloids. Caffeine and theobromine are naturally occurring purine alkaloids. They are widely consumed through foods such as tea, coffee, cola, and chocolate. Caffeine was the predominant alkaloid, representing 99% to the total (Table 3). Significant differences ($p < 0.05$) in caffeine and theobromine existed between low- and high-quality black teas. The USDA[30] reported the content of caffeine in instant tea as 3680 mg/100 g. The caffeine levels found in instant teas were higher than those published in the literature for different types of black tea (1525–3900 mg/100 g).[6,38−40] A small amount of theobromine was also found in both instant teas (37–44 mg/100 g). A variety of adverse health effects have been attributed to alkaloid consumption, including behavior abnormalities, hypertension, and hypercholesterolemia, although inconsistently.[41]

Sugars. Three sugars were positively identified in instant teas; these included fructose, glucose, and sucrose. Significant variations ($p < 0.05$) in fructose, glucose, and sucrose were observed between low- and high-quality instant teas. The total sugar content varied between 12.70 and 12.71 g/100 g, and no significant differences ($p > 0.05$) existed between the two samples (Table 4). Among identified sugars, fructose represented about 55–60% of the total amount, followed by glucose at 26–33% and sucrose at 8–20%. The total sugar content in the present study was higher than that reported in the literature (5.53 g/100 g).[30] The observed difference may be due to either variety or processing conditions.

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).[42] Alasalvar et al.[9] positively identified five sugars (fructose, galactose, glucose, sucrose, and xylose) in seven grades of black tea, and the total sugar content ranged from 2.51 to 3.59 g/100 g. Galactose and xylose were not detected in the present study.

Organic Acids. Five organic acids (citric, fumaric, malic, oxalic, and tannic) were positively identified in instant teas (Table 4). Some variations ($p < 0.05$), albeit to different extents, were observed among organic acids in low- and high-

Table 4. Taste-Active Compounds of Instant Teas (Values per 100 g of Instant Tea)[a]

	unit	instant tea produced from	
		low quality	high quality
sugars			
fructose	g	7.62 ± 0.02a	6.92 ± 0.12b
glucose	g	4.14 ± 0.01a	3.30 ± 0.08b
sucrose	g	0.95 ± 0.01a	2.48 ± 0.00b
total	g	12.71 ± 0.04a	12.70 ± 0.20a
organic acids			
citric	g	1.18 ± 0.12a	0.83 ± 0.04a
fumaric	g	0.01 ± 0.00a	0.01 ± 0.00b
malic	g	2.07 ± 0.11a	2.32 ± 0.07a
oxalic	g	1.79 ± 0.02a	2.00 ± 0.03b
tannic[b]	g	21.89 ± 0.27a	20.43 ± 0.49a
total		26.94 ± 0.27a	25.58 ± 0.42a
free amino acids			
alanine	mg	92.7 ± 8.9a	105.1 ± 9.5a
arginine	mg	231.9 ± 26.6a	255.4 ± 15.8a
asparagine	mg	78.6 ± 10.3a	76.3 ± 3.9a
aspartic acid	mg	209.7 ± 14.5a	181.8 ± 0.7a
glutamic acid	mg	241.1 ± 7.3a	219.9 ± 22.8a
glutamine	mg	97.2 ± 0.5a	56.6 ± 4.2b
glycine	mg	4.0 ± 0.5a	1.6 ± 0.7a
histidine	mg	14.5 ± 2.2a	14.8 ± 1.5a
isoleucine	mg	49.7 ± 3.0a	29.0 ± 1.4b
leucine	mg	63.3 ± 7.1a	41.3 ± 1.9b
lysine	mg	32.2 ± 3.2a	21.1 ± 1.0b
phenylalanine	mg	69.8 ± 9.3a	29.0 ± 3.2b
proline	mg	46.0 ± 2.0a	27.6 ± 1.1b
serine	mg	109.3 ± 14.1a	87.1 ± 6.3a
theanine	mg	572.2 ± 63.6a	512.9 ± 24.4a
tryptophan	mg	54.7 ± 13.0a	34.0 ± 5.1a
tyrosine	mg	17.5 ± 1.7a	10.2 ± 0.1b
valine	mg	25.1 ± 0.2a	15.4 ± 0.5b
total	mg	2010 ± 183a	1719 ± 90a

[a]Data are expressed as the mean ± SD ($n = 3$) on a fresh weight basis. Means followed by the same letter, within a row, are not significantly different ($p > 0.05$). [b]Commercially available tannic acid reference compound was found to contain three derivatives, all of which were detected in tea samples and quantified as their sum to express the total tannic acid.

quality instant teas. Among identified organic acids, tannic acid was most abundant in both teas, representing 80−81% of the total organic acids present. Various organic acids have been reported in different types of black teas at varying concentrations.[9,34,43−46] Tannic acid has a characteristic astringent taste and could be responsible for the astringent taste together with theaflavins and thearubigins in instant teas.[9,47] Tannic acid has also been reported as being a predominant organic acid in seven grades of black tea.[9]

Free Amino Acids. A total of 18 free amino acids were identified and quantified in instant teas (Table 4). Both instant teas contained large amounts of theanine (512.9−572.2 mg/100 g), arginine (231.9−255.4 mg/100 g), glutamic acid (219.9−241.1 mg/100 g), and aspartic acid (181.8−209.7 mg/100 g). These four free amino acids constituted an average of 62−68% of the total free amino acids. Although significant variations ($p < 0.05$) were observed for the concentration of individual free amino acid, with some exceptions, between instant teas, total free amino acid content was insignificant ($p > 0.05$). Despite the fact that all 18 free amino acids contribute a characteristic taste to the flavor of the instant teas, theanine deserves special attention due to its beneficial health benefits[48−50] as well as umami taste. Theanine was the most abundant free amino acid in both instant teas, representing around 29−30% of the total free amino acids. Theanine has also been reported as being a predominant free amino acid in seven grades of black tea.[9]

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.

The present work suggests that some variations, albeit to different extents, were observed between the instant teas produced from low- and high-quality black tea. The combination of macronutrients, micronutrients, and phytochemicals in instant tea makes it a great candidate for functional beverage application. The instant teas could not be distinguished on the basis of their compositional, nutritional, and functional characteristics or taste-active compounds. Therefore, further research is needed to observe the possible differences between the two using aroma-active compounds.

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