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ORIGINAL PAPER

Comparison of the Nutrient and Chemical Contents of Traditional Korean Chungtaejeon and Green Teas

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Abstract This study was conducted in order to compare the nutrient and chemical contents of two Korean teas: traditional Chungtaejeon tea (CTJ) with that of green tea (GT). Main bioactive compounds and the antioxidant activities using four radical scavenging assays (ABTS, CUPRAC, FRAP and DPPH) in methanol and acetone extracts of both teas were determined. It was found that the contents of vitamin C, amino acids and total nitrogen in CTJ were lower than that of GT ($p<0.05$). Caffeine, reducing sugar and chlorophyll contents in CTJ were similar to GT. Catechin (C), epicatechin (EC), and epigallocatechin (EGC) contents were lower in CTJ than in green tea ($p<0.05$), but gallocatechin (GC), epicatechin gallate (ECG), epigallocatechin gallate (EGCG) and catechin gallate (CG) showed no significant differences between CTJ and GT ($p \geq 0.05$). The contents of polyphenols, flavonoids, flavanols and tannins in CTJ methanol were 229.30±11.3 mg GAE/g dry weight (DW), 15.24±0.8 mg CE/g DW, 109.10±5.1 mg CE/g DW and 25.68±1.2 mg CE/g DW, respectively, and significantly higher than in acetone extracts ($p<0.05$). Flavonoids (quercetin and kaempferol) were higher in GT than in CTJ and myricetin was higher in CTJ ($p<0.05$). Threonine and aspartic acid was lower, and glutamic acid was higher in CTJ compared with GT ($p<0.05$). Free amino acid content in CTJ and GT showed no significant difference. Potassium and magnesium in CTJ were lower compared to GT, but no significant difference was found for iron, manganese and calcium. Also, the level of the antioxidant activity by all four used assays was significantly higher in CTJ and in methanol was higher than in acetone extracts ($p<0.05$). In conclusion, traditional fermented Korean tea Chungtaejeon contains high quantities of bioactive compounds and possesses high antioxidant activity. The contents of the bioactive compounds and the levels of antioxidant activities are significantly higher in methanol than in acetone extracts. 3-D fluorescence and FTIR- spectroscopy showed slight differences between the two investigated tea samples and can be used as additional tools for identification of polyphenols. Both studied teas can be recommended as a source of bioactive compounds.

Keywords Korean teas · Bioactive substances · Antioxidant activity

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Introduction

It has been previously established that both black and green teas contain similar amounts of flavonoids, however, the difference is in their chemical structure: green tea contains more catechins (simple flavonoids), while the oxidation undergoes by the leaves in order to make black tea to convert these simple flavonoids into theaflavins and thearubigins [1].

The traditional Korean tea Chungtaejeon contains relatively high quantities of nutrient and chemical components: microelements, flavonoids (quercetin, kaempferol and myricetin), catechin, epicatechin, epigallocatechin and tannin [2]. It was of great interest to know if this natural product as other kinds of teas exercises high bioactivities. There are high amounts of bioactive compounds and high antioxidant activities in different herbal teas [3–9].

Tea is the most widely consumed beverage in the world, second only to water. Green tea is healthier than black tea due to the low incidence of heart disease and cancer [10]. Some investigators claim that different teas as part of diet prevent myocardial infarction [8–11], diabetes [12] and cancer [13]. The protein, amino acid and mineral compositions of teas are important for their consumption [14, 15].

Underlying mechanisms for the beneficial effects of tea include vasculoprotective, antioxidative, antithrombogenic, antiinflammatory, and lipid-lowering properties of tea flavonoids. Although promising experimental data on beneficial effects of tea in various cardiovascular diseases are available [8–11], new varieties of tea samples are important. As it was shown below there are a number of reports on different tea varieties, excluding the wild Korean tea. Therefore, the aim of this report was to compare the less investigated CTJ with well known GT.

As far as we know, no results of such comparative investigation were published.

Materials and Methods

Chemicals

All chemicals were purchased from Sigma-Aldrich (Sigma Chemical Co., St. Louis, MO, USA).

Plant Materials

Chungtaejeon (CTJ) was prepared from wild leaves, which were collected from the mountain in Chung-heoung county, Korea, in June, 2008. The production methods were adopted from Korean standard processing. Green tea (GT) leaves were harvested from the local farm of Bo-seong county, Korea, and collected at the same time as CTJ and were processed as a roasted tea product. Lyophilized tea samples were extracted at room temperature with methanol (^{Met}) in concentration of 25 mg/mL and acetone (^{Acet}) in concentration of 40 mg/mL (Tables 1 and 2).

Methods

Vitamin C was carried out by HPLC system (Waters, Model 515, USA) with a mobile phase of tridecylammonium, water and formic acid [2]. Total amino acids were analyzed using ninhydrin assay [16] spectrophotometrically (Spectrophotometer Hewlett Packard, Model 8452A, Rockville, USA). Free amino acids were simultaneously separated in a HPLC system, using mobile phases of lithium citrate buffer with different pHs. Total nitrogen was done by the Kjeldahl method [17]. Caffeine analysis was carried out by HPLC (Waters, Model 515, USA), with a mobile phase of methanol/water [18]. Reducing sugars were extracted with 80 % ethanol, following fermentation [2]. Chlorophyll was determined in acetone extracts [19]. Catechins were analyzed by HPLC system (Waters, Model 515, USA) at 254 nm with a mobile phase consisting of acetonitrile, ethyl acetate, and 0.05 % phosphoric aqueous solution [20, 21]. Flavonoids were extracted with 25 mL of 50 % ethanol containing 0.3 mg of morin and 20 mg of tert-butylhydroquinone with mobile phases of 1 % formic acid and acetonitrile.

Minerals were determined by wet ashing of inorganic content (Zn, Mn, Fe, Mg and K) and measured by an atomic absorption spectrophotometer (Avanta, GBC, Australia) [22].

Tannins were measured at 500 nm using 4 % methanol vanillin solution with (+)-catechin as a standard [23]. The contents of total polyphenols, flavonoids, flavanols and antioxidants were determined as previously described [23–26].

Four complementary assays were used: 1) Ferric Reducing Antioxidant Power (FRAP); 2). 2, 2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diamonium salt (ABTS.+); 3). 1-Diphenyl-2-picrylhydrazyl method (DPPH); 4). Cupric reducing antioxidant capacity (CUPRAC) [23–26].

Three-dimensional fluorescence (3-D FL) spectra for tea extracts in methanol and acetone at a concentration of 0.005 mg/mL and 0.02 mg/mL, respectively, were recorded on a model FP-6500, Jasco spectrofluorometer, serial N261332, Japan [27]. The presence of polyphenols (flavonoids and phenolic acids) in the investigated samples was studied by Fourier transform infrared (FT-IR) spectroscopy [23] with a Bruker Optic GMBH Vector FT-IR spectrometer (Bruker Optic GMBH, Attingen, Germany).

Results and Discussion

In 3-D FL spectra [the excitation and the emission wavelengths and the fluorescence intensity (FI)] were used as the axes in order to investigate the information of the tea samples. The contour maps, as the elliptical shapes, provided additional information (Fig. 1, position A, CTJ methanol extract).

Table 1 Some bioactive compounds extracted with methanol (^{Met}) and acetone (^{Acet}) from Chungtaejeon (CTJ) and green (GT) teas

Samples	Polyphenols, mg GAE/g	Flavonoids, mg CE/g	Flavanols, mg CE/g	Tannins, mg CE/g
CTJ ^{Met}	229.30±11.3^{b}	15.24±0.8^{b}	109.10±5.1^{b}	25.68±1.2^{b}
CTJ ^{Acet}	8.92±0.5^{a}	0.37±0.01^{a}	2.17±0.1^{a}	0.97±0.05^{a}
GT ^{Met}	142.14±9.5^{b}	25.26±1.3^{b}	69.02±3.3^{b}	23.68±1.1^{b}
GT ^{Acet}	4.66±0.2^{a}	1.26±0.1^{a}	0.83±0.06^{a}	0.78±0.05^{a}

Values are means ± SD per g dry weight (DW) of five measurements

Values in columns with different superscript letters are significantly different (p<0.05)

The result showed that contour maps of methanol extracts of Chungtaejeon tea [Fig. 1, Aa (two peaks at: ex/em 275/310 nm with FI of 893 and ex/em 275/610 nm with FI of 229)]; and green tea [Fig. 1, Ba (two peaks at: ex/em 275/310 nm with FI of 1,000 and ex/em 275/610 nm with FI of 268)] were similar with insignificant differences of the intensity units.

The acetone extracts were slightly different [Fig. 1, Ab (peaks at: ex/em 350/390 nm with FI of 166 and a small one at ex/em 350/670 nm with FI of 18.9)]; and Bb [(not shown; peak at: ex/em 350/390 nm with FI of 113)].

The wavelengths numbers of FTIR spectra for catechin at 827, 1,039, 1,115, 1,143, 1,286, 1,478, 1,511 and 1,610 cm^{-1} were assigned to C-H alkenes, -C-O alcohols, C-OH alcohols, -OH aromatic, C-O alcohols, C-H alkanes, C = C aromatic ring, and C = C alkenes. Gallic acid showed the following wavelengths numbers (cm^{-1}) of 866, 1,026, 1,238, 1,450, 1,542 and 1,618. CTJ and GT samples (Fig. 2, curves 1 and 2) in the region of polyphenols showed slightly different bands than the standards, but the wavelengths of absorption bands (cm^{-1}) were similar in this group of two teas (Fig. 2): at 1697.4, 1638.3, 1454.7, 1362.9, 1238.3, 1041.8 and 1146.5.

Table 2 The antioxidant activity (μM TE/g DW) of Chungtaejeon (CTJ) and green (GT) teas extracted with methanol (^{Met}) and acetone (^{Acet})

	CTJ	GT
ABTS ^{Met}	2087.56±61.3^{b}	1276.77±52.8^{b}
ABTS ^{Acet}	55.53±5.3^{a}	41.86±3.8^{a}
CUPRAC ^{Met}	852.0±32.5^{b}	668.19±273.7^{b}
CUPRAC ^{Acet}	60.45±3.3^{a}	24.54±0.8^{a}
FRAP ^{Met}	19.37±1.3^{b}	9.37±0.8^{b}
FRAP ^{Acet}	0.75±0.3^{a}	0.31±0.2^{a}
DPPH ^{Met}	112.96±4.5^{b}	72.98±3.6^{b}
DPPH ^{Acet}	9.83±0.4^{a}	4.09±0.2^{a}

Values are means ± SD of five measurements. Values in columns with different superscript letters are significantly different (p<0.05). Abbreviations: ABTS.$^{+}$; 2, 2-azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diamonium salt; CUPRAC, cupric reducing antioxidant capacity; DPPH, 1-diphenyl-2-picrylhydrazyl method; FRAP, ferric reducing ability of plasma; TE, Trolox equivalent. DW, dry weight

The contents of vitamin C, amino acids and total nitrogen in CTJ were lower than that of GT (Fig. 3a, p< 0.05). Caffeine, reducing sugar and chlorophyll contents in CTJ were similar to GT (Fig. 3a). Bioactive compounds (polyphenols, flavonoids, flavanols and tannins, Table 1) were significantly higher in methanol extract of CTJ (229.30±11.3 mg GAE/g DW, 15.24±0.8 mg CE/g DW, 109.10±5.1 mg CE/g DW and 25.68±1.2 mg CE/g DW) than in acetone (p<0.05). Also, other authors reported comparable results [15]. It was found considerable variability in both total phenols (80.5–134.9 mg/g DW in black teas and 87–106.2 mg/g DW in green teas) and catechins (5.6–47.5, 51.5–84.3, and 8.5–13.9 mg/g DW in black, green, and fruit teas, respectively). These data [13, 16] were comparable with our results. The contents of catechins were 11 and 16 mg/g DW in CTJ and GT, respectively. Caffeine contents of black teas (22–28 mg/g DW) were significantly higher than in less fermented green teas (11–20 mg/g DW) (Fig. 3a). The relative concentration of the five major tea catechins ranked EGCG > ECG > EC > EGC > C in comparison with our results: EGC > ECG > GCG > EC > GC > C > EGCG > CG in GT and EGC > GC > ECG > GCG > EC > C = EGCG > CG in CTJ. These results are in agreement with data of others [13]. According to our results, the highest content was of EGC for both teas and the lowest of CG. Another EG constituent, which is higher in GT as much as twice [11, 21] is active in the protection of LDL and vascular endothelium. In addition to seven catechins [21] another catechin was determined in the two teas. The amount of total flavonoids was higher in GT and flavanols in GTJ. Total catechins (Fig. 3b) were in the range (5.01–5.69 g/100 g) and were slightly lower than reported by Krawczyk and Druzynska [20]. The obtained results depend on the extraction procedure, therefore the variety of data was found. The biologically active components of CTJ include polyphenols and a number of catechins, epicatechin, epicatechin-3-gallate, epigallocatechin, and epigallocatechin-3-gallate. As it was mentioned previously these constituents in tea are biologically active and the most important for cardiovascular prevention [9–11]. Since traditionally fermented teas were

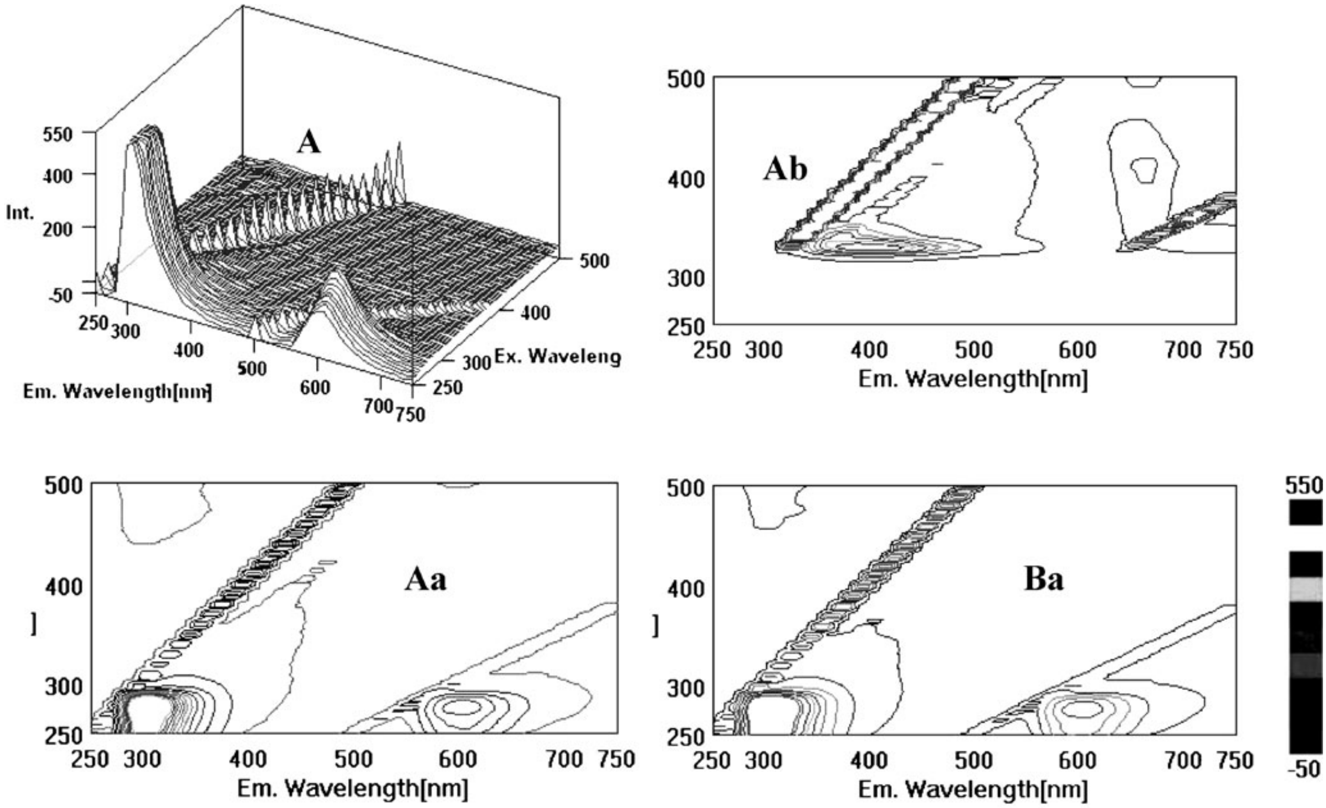
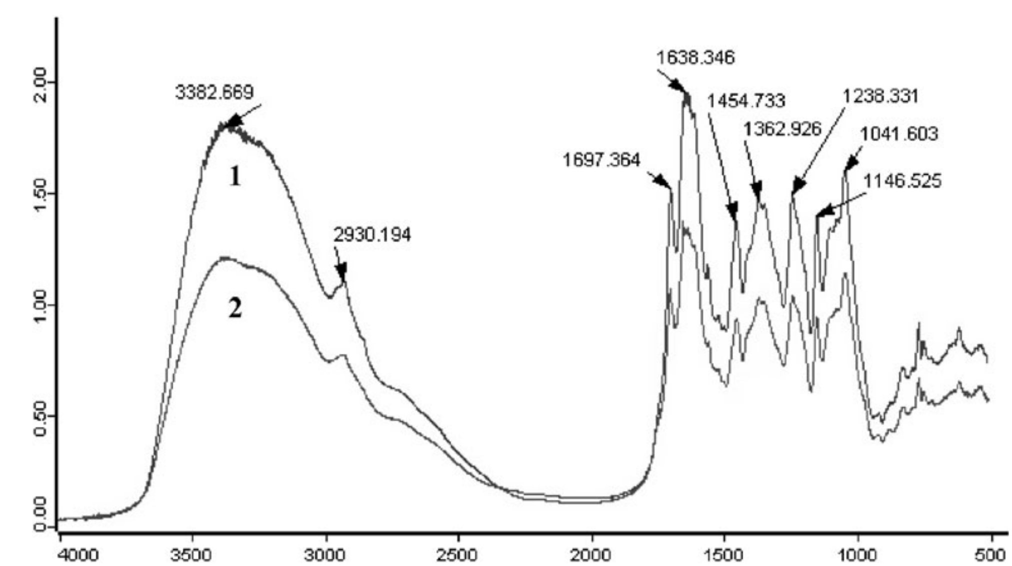


Fig. 1 Three dimensional fluorescence map of 0.005 mg/mL of methanol extracts of Chungtaejeon tea (CTJ, A). The contour maps (Aa and Ba) displayed a view of the corresponding fluorescence spectra of 0.005 mg/mL of methanol extracts of CTJ and green teas; and (Ab) of 0.02 mg/mL of acetone extracts of Chungtaejeon tea. Abbreviations: A on axis Z: Int., fluorescence intensity; X: Em. Wavelength, emission wavelength; Y: Ex. wavelength, excitation wavelength, Aa, Ba, Ab, axis X: Em. Wavelength, emission wavelength; Y, excitation wavelength; the fluorescence intensity values from -50 to 550 are presented

formerly considered to be inferior to green tea in fighting against oxidative stress—related diseases but later found to raise the plasma antioxidant levels at the same rate as green tea does, this finding of practically unchanged GC, ECG, EGCG and CG levels in the two tea varieties may be important for human health associated with food research.

The mineral contents of CTJ were similar with the data of other authors, where the extraction rates for K (71 %),

Fig. 2 FTIR spectra of Chungtaejeon (1) and green (2) tea samples



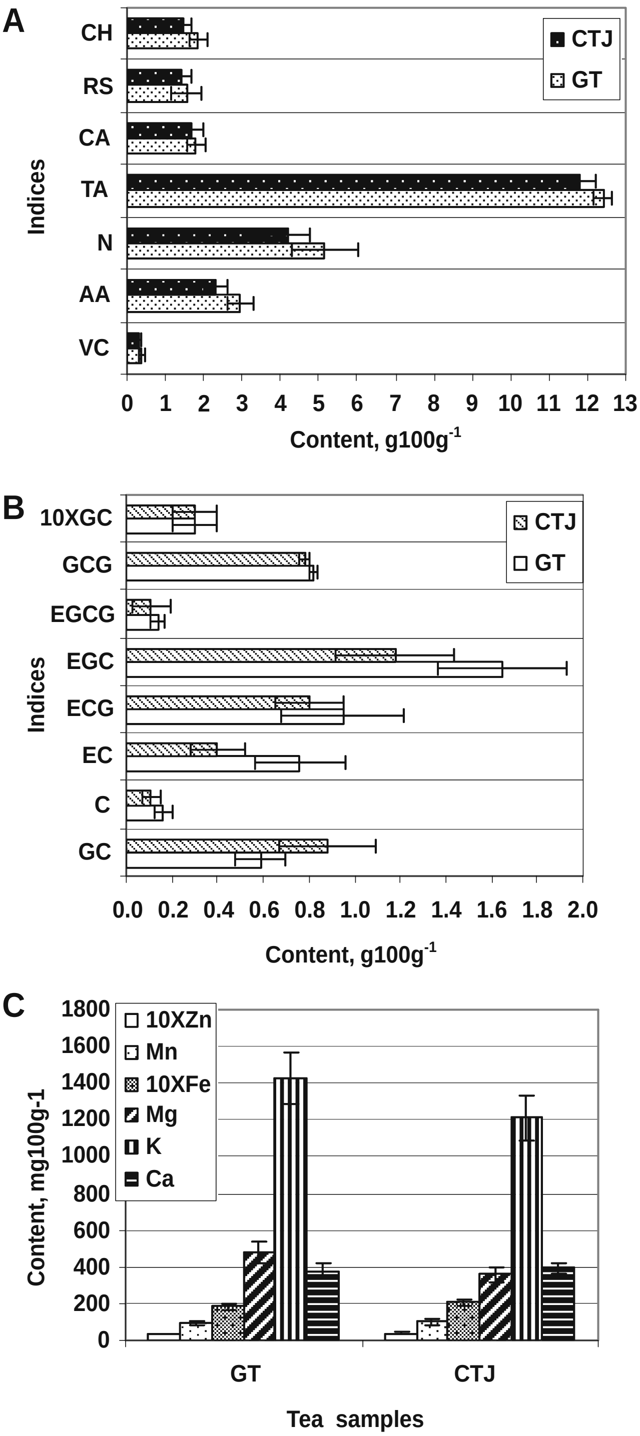


Fig. 3 Partial characterization of Chungtaejeon tea (CTJ) and green tea (GT). A, Indices of Vitamin C (VC), Reducing sugars (RS), Caffeine (CA), Tannin (TA), Total nitrogen (N), Total amino acids (AA), Chlorophyll (CH); B, Contents of catechins in Chungtaejeon and green tea: 10XGallocatechin (10XGC), Gallocatechin gallate (GCG), Epigallocatechin gallate (EGCG), Epigallocatechin (EGC), Epicatechin (EC), Catechin (C), Epicatechin gallate (ECG), Catechin gallate (CG); C, Contents of minerals

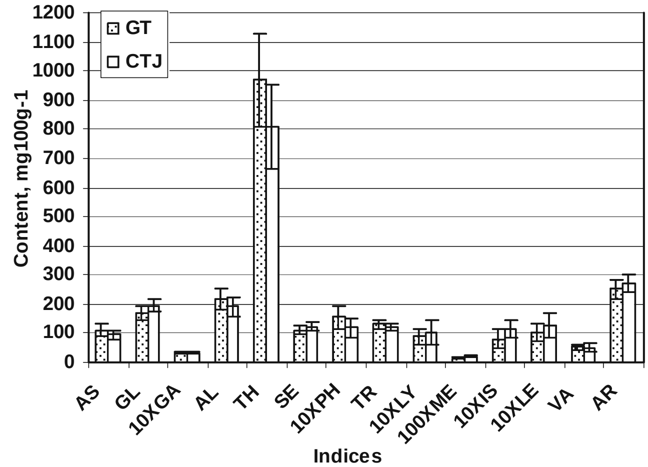


Fig. 4 Amino acid content in Chungtaejeon and green tea, teas. AS, Aspartic acid; GL, Glutamic acid; 10XGA, Gaba; AL, Alanine; TH, Threanine; SE, Serine; 10XPH, Phenylalanine; 10XPH, Phenylalanine; TR, Tryptophan; 10XLY, Lysine; 100XME, Methionine; 10XIS, Isoleucine; 10XLE, Leucine; VA, Valine; AR, Arginine

Mg (38 %) [22], and in our case K was as much as twice higher than Mg. The contents of Mn and Fe were comparable (Fig. 3c). Therefore, potassium and magnesium in CTJ were lower compared to GT, but no significant differences were found for iron, manganese or calcium contents.

The nutritional composition of investigated teas was similar in iron content [14] and caffeine [18]. Free and total amino acid contents in CTJ and GT showed no significant differences (Fig. 3a). Threonine and aspartic acid was lower, and glutamic acid was higher in CTJ compared with GT (Fig. 4, $p<0.05$). The level of the antioxidant activity by all four used assays (ABTS, CUPRAC, FRAP and DPPH) was significantly higher in methanol extract of Chungtaejeon (Table 2) than in acetone ($p<0.05$). The results were similar to others [5, 28].

Antioxidant capacities assayed by the four electron transfer (ET)—based methods were found to be higher in methanol than in acetone, probably due to facilitated e-transfer in ionizing solvents capable of anion (phenolate) solvation, because methanol is the alcohol that best supports phenol ionization. Thus, the assayed tea extracts in methanol having a greater ratio of phenolate-to-phenol showed higher antioxidant activity than in acetone [29]. For the same sample in the same solvent, FRAP values were significantly lower than those of CUPRAC (Table 2), primarily because the FRAP test has been reported to yield incomplete oxidation reactions with polyphenolic antioxidants within the protocol time period of the assay, while CUPRAC oxidation is complete within 1.5 h for most common antioxidants, due to the faster reaction kinetics of d9-cupric ion in preference to half-filled (and therefore inert in high-spin state) d5-ferric ion [30].

Conclusions

1. Traditional fermented Korean tea Chungtaejeon contains high quantities of bioactive compounds and has high antioxidant activity. 2. Both studied teas can be recommended as potent bioactive plants.

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