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Full Length Research Paper

Effect of deep sea water on the antioxidant activity and catechin content of green tea

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Application of deep sea water (DSW) on preparation of green tea was evaluated. Green tea leaves were soaked in desalinated deep sea water (DSW) at 75°C for 10 min, and were evaluated for antioxidant activity and catechin content. DSW green tea showed higher antioxidant activity than green tea prepared with distilled water (DW). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities and reducing power of DW and DSW green teas were 57.88 and 83.98%, and their optical densities were 0.99 and 1.14, respectively. Nitrite scavenging activity was also higher in DSW green tea than in DW green tea. Overall amounts of catechins and caffeine were also greater in DSW green tea. For example, epigallocatechin gallate content in DW and DSW green teas were 13.20 and 17.97 mg/g, respectively. However, there was no significant colour difference between the two tea preparations. These results indicated that the chemical properties of green tea are significantly affected by DSW and that DSW results in high quality green tea.

Key words: Green tea, deep sea water, antioxidant activity, catechins.

INTRODUCTION

Green tea is one of the most popular beverages consumed worldwide. Many published studies have reported on the biological and biochemical properties of green tea and its catechins, such as antioxidative, anticarcinogenic, antimicrobial, antidiabetic, heavy metal removal, and detoxification properties. Moreover, green tea has shown to prevent the progression of cardiac disorders, hypertension, and obesity (Yamanishi et al., 1995; Ahmad et al., 1999). The main components of green tea are polyphenols, fibres, proteins, carbohydrates, fat, peptides, caffeine, minerals, and organic acid (Sato and Miyata, 2000). Green tea contains catechins, which low molecular weight polyphenols are belonging to the flavan-3-ol class of flavonoids (Graham, 1992). The catechins comprise a family of four major substances - epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), and epigallocatechin gallate (EGCG) - and four minor catechins - catechin (C), catechin gallate (CG), gallocatechin (GC), and gallocatechin gallate (GCG), all epimers of the major catechins (Graham 1992). The variety, season, species, soil and weather conditions, growing environment, manufacturing method, and extraction solvent and particle size of the tea leaves influence the composition of tea and the final infusion (Oh et al., 1995; Astill et al., 2001). Moreover, soaking time and temperature during the preparation of green tea influence the polyphenol content in tea (Gulati et al., 2003; Wang et al., 2003).

Deep sea water (DSW) generally refers to seawater obtained at a depth of more than 200 m. DSW contains abundant essential minerals such as potassium, calcium, magnesium, sodium, and selenium, along with minute amounts of many trace elements such as iron, copper, zinc, manganese, and chromium, which are not found in the common surface water (Nakagawa et al., 2001). DSW has been applied in the processing of cosmetics;

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Abbreviations: DSW, Deep sea water; **DW,** distilled water; **DPPH,** 2,2-diphenyl-1-picrylhydrazyl; **EC,** epicatechin; **ECG,** epicatechin gallate; **EGC,** epigallocatechin; **EGCG,** epigallocatechin gallate; **C,** catechin; **CG,** catechin gallate; **GC,** gallocatechin; **GCG,** gallocatechin gallate; **NSA,** Nitrite scavenging ability.

health foods (e.g. mineral water, fermentation foods, soft drinks, bean-curd, salted and dried fish etc.); and marine, agricultural, and medical care products. DSW salt has also been reported to possess antimutagenic and cytotoxic properties (Ham et al., 2008). In this study, the quality of green tea prepared with DSW was evaluated for its antioxidant activity and catechin composition.

MATERIALS AND METHODS

Reagents

In this study, the authors used commercial desalinated DSW (Ulleung Mine Water, Cheiljedang Co., Seoul, Korea). Eight catechin standards and caffeine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Methanol and 1,1-diphenyl-2-picrylhydrazyl (DPPH), were also purchased from Sigma Chemical Co. All remaining chemicals were of analytical grade and were used as received. The water used in HPLC was prepared with a super purity water system (Purite Ltd., Oxon, UK).

Preparation of green tea

Commercially, processed green tea leaves (Sulloc Green Tea Leaves, Camellia sinensis var. sinensis), which were originally harvested in April 2007, in Cheju, Korea, were purchased from Amorepacific Co. (Seoul, Korea). Green tea leaves (1.0 g) were extracted for 10 min with 100 mL of DW or DSW at 75°C. Then, the extracts (green tea) were filtered using a filter paper and immediately used for the following experiments.

DPPH radical scavenging activity

The antioxidant activity of the two green tea extracts was determined by the DPPH radical scavenging activity (Blois, 1958). After mixing 0.1 mL of green tea extract with 0.9 mL of 0.041 mM DPPH radical in ethanol for 30 min, the absorbance was measured at 517 nm by using a UV-VIS spectrophotometer (UV 1601, Shimadzu Co. Ltd., Kyoto, Japan). The radical scavenging activity was expressed as a percentage according to the following formula:

% DPPH radical scavenging activity = [1- (sample OD/control OD)] × 100

Reducing power

The reducing power of each green tea extract was determined according to the method described by Oyaizu (1986). The green tea extract (1 mL, 1 mg/mL), phosphate buffer (1 mL, 0.2 M, pH 6.6), and potassium ferricyanide (1.0 mL, 10 mg/mL) were mixed and incubated at 50°C for 20 min. Trichloroacetic acid (1.0 mL, 100 mg/mL) was added to the mixture and centrifuged at 13,400 × *g* for 5 min. The supernatant (1.0 mL) was mixed with distilled water (1.0 mL) and ferric chloride (0.1 mL, 1.0 mg/mL), and the absorbance was measured at 700 nm.

Nitrite scavenging ability (NSA)

The NSA of green tea was determined by using Griess reagent (Kato et al., 1987). First, 1 mL of each green tea extract was mixed with 1 mL of 1 mM sodium nitrite. The mixture was added to 8 mL of 0.2 M citrate buffer (pH 3.0 and 4.2) and incubated for 1 h at 37°C. Then, 1 mL aliquot was removed and added to 2 mL of 2% acetic

acid and 0.4 mL of Griess reagent (1% sulfanilic acid and 1% naphthylamine in a methanol solution containing 30% acetic acid). After vigorous vortex mixing, the mixture was placed at room temperature for 15 min and the absorbance was measured at 520 nm. The NSA (%) was calculated by the following equation.

NSA (%)=[1 - (A – C)/B)]×100

Where, A is the absorbance of treated sample, C is the absorbance of green tea, and B is the absorbance of 1 mM $NaNO_2$.

Colour analyses

Colour analyses were carried out using a colorimeter (Chroma-meter-2 Reflectance, Minolta, Osaka, Japan) equipped with a CR-200 measuring head. The instrument was standardized against a white tile before each measurement. The colour was expressed in 'L' [lightness, 0~100 (100=white, 0=black)], 'a' [redness, -60~+60 (-60=green,+60=red)], and 'b' [yellowness, -60~+60 (-60=blue,+60=yellow)] hunter scale parameters (Mastrocola and Lerici, 1991).

Catechin analysis

The levels of catechins and caffeine in green tea were measured by HPLC (Lee et al., 2006). The HPLC systems consisted of LC-6AD pumps (Shimadzu Co. Ltd.) in a two-pump gradient system, an SPD-10AVP UV-vis detector, an SIL-10ADVP auto sample injector, and a CTO 10AVP column oven. The column was a Shim-pack VP ODS (5 μm, 250 × 4.6 mm, Shimadzu Co. Ltd.), equipped with a Shi-pack CLC guard column (10 × 4 mm, Shimadzu Co. Ltd.). Mobile phases were (A) 0.1% orthophosphoric acid in water (v/v) and (B) 0.1% orthophosphoric acid in methanol (v/v). The gradient was as follows: 0 - 5 min, 40% B; 5 - 12 min, linear gradient from 40 - 50% B; 12 - 27 min, 50% B; 27 - 30 min, linear gradient from 50 - 20% B; 30 - 35 min, linear gradient 20 - 0% B. The post-run time was 5 min. Elution was performed at a solvent flow rate of 1 mL/min. Detection was accomplished with a UV-vis detector, and chromatograms were recorded at 210 nm. The column was maintained at 40°C. The sample injection volume was 10 μL. The peaks were identified by comparing their retention times with authentic standards. The concentration range of authentic catechins and caffeine for the standard curve was 1.00 - 0.01 mg/mL.

Statistical analysis

Each experiment was performed in triplicate, and all measurements were analyzed in 3 independent runs. Analyses of variance were conducted by the General Linear Model procedure using SAS software (SAS Institute, 1995). Student-Newman-Keul's multiple range tests were used to test for significant differences between the mean values for each treatment ($p < 0.05$).

RESULTS AND DISCUSSION

DPPH radical scavenging activity

Green tea has been reported to possess antioxidant activity. Antioxidant activity of DSW green tea was compared with that of DW green tea by evaluating their radical scavenging activity and reducing power. DPPH

Table 1. DPPH radical scavenging activity, reducing power, and nitrite scavenging activity of DW and DSW green teas.

	Green tea samples[1)]	
	DW green tea	**DSW green tea**
DPPH RSA (%)	57.88 ± 2.00[b2)]	83.98 ± 1.39[a]
RP (Optical density)	0.99 ± 0.01[b]	1.14 ± 0.01[a]
NSA (pH 3.0)	97.01± 0.05[b]	97.15 ± 0.02[a]
(pH 4.2)	31.33 ± 0.05[b]	37.12 ± 0.42[a]

[1)]DW, distilled water; DSW, deep seawater. [2)]Different letters (a and b) within a row are significantly different ($p < 0.05$); n = 3. DPPH RSA, 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity; RP, reducing power; and NSA, nitrite scavenging ability.

Table 2. Hunter colour L, a, and b values of green tea extracts.

Green tea samples	Colour value[1)]			
	L	**a**	**b**	**ΔE[2)]**
DW green tea	94.74 ± 0.48[a3)]	-2.27 ± 0.06[a]	13.38 ± 0.13[b]	0
DSW green tea	94.69 ± 0.01[b]	-2.16 ± 0.04[b]	13.64 ± 0.03[a]	0.04

[1)]L, degree of lightness; a, degree of redness; b, degree of yellowness; ΔE, overall colour difference. [2)]$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$. [3)]Different letters (a and b) within a column are significantly different ($p < 0.05$); n = 3.

radical has been widely used to determine antioxidant activity because of its simplicity and high reproducibility. As shown in Table 1, DSW green tea (83.98 ± 1.39%) showed more than 1.45-fold higher DPPH radical scavenging activity than DW green tea (57.88 ± 2.00%).

Reducing power

The antioxidant ability of certain compounds is associated with their reducing power, thus the reducing power may serve as a significance indicator of potential antioxidant activity. The reducing power of DSW green tea was 1.14 ±0.01 (absorbance value), which was about 15% higher than that of DW green tea (0.99±0.01 absorbance value) (Table 1).

NSA

Nitrite ions in the acidic environment of the stomach induce mutagenic and cell-damaging reactions (Kato and Puck, 1971). EGCG in green tea is an efficient inhibitor of *N*-nitrosation. The effect of DSW on the NSA of green tea was evaluated. As shown in Table 1, both DW and DSW green tea exhibited very high NSA (more than 97%) at pH 3.0. At pH 4.2, DSW increased the NSA of green tea from 31.33 ± 0.05 to 37.12 ± 0.42% compared to DW green.

Green tea contains polyphenols, which include flavanols, flavandiols, flavonoids, and phenolic acids. Polyphenols are the most biologically active components of tea, and flavanols are the main polyphenol compounds in green tea. Flavanols have received much attention because of their pharmaceutical properties such as antioxidative, antitumour, and anticarcinogenic properties (Sakanaka et al., 1989; Chung et al., 1992; Chung et al., 1998). DSW increased the antioxidant activity of green tea, as evidenced by its DPPH radical scavenging activity and reducing power (Table 2). The increased level of radical scavenging activity (45%) and reducing power (15%) differed significantly. Reducing power was determined by measuring the reduction of Fe^{+3} in the Fe^{+3}/ferricyanide complex to Fe^{+2}. The antioxidant activities of putative antioxidants have been attributed to various mechanisms, including prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, and radical scavenging (Diplock, 1997). Therefore, there may not always be a linear correlation between radical scavenging activity and reducing power activity.

DSW also increased the NSA of green tea. Nakagawa and Yokozawa (2002) observed that the galloyl group enhances the nitric oxide scavenging activity of tannin, whereas caffeine does not affect nitric oxide production.

Table 3. Catechins and caffeine contents of DW and DSW green teas (Unit: mg/g).

Green tea sample	Catechins[1)]							Caffeine
	EC	ECG	EGC	EGCG	GC	GCG	Total	
DW green tea	5.44±0.25[b2)]	2.01±0.45[b]	19.73±0.43[b]	13.20±3.76[b]	0.77±0.03[b]	0.77±0.58[a]	41.92	13.14±0.34[b]
DSW green tea	6.28±0.01[a]	2.59±0.04[a]	21.82±0.04[a]	17.97±2.75[a]	0.92±0.00[a]	0.56±0.07[a]	50.14	15.42±0.02[a]

[1)]EC, epicatechin; ECG, epicatechingallate; EGC, epigallocatechin; EGCG, epigallocatechin-3-gallate; GC, gallocatechin; and GCG, gallocatechingallate. [2)]Different letters (a and b) within a column are significantly different ($p < 0.05$); n = 3.

The amount of catechins containing galloyl groups, ECG and EGCG, in DSW green tea increased from 2.01±0.45 to 2.59±0.04 mg/g and from 13.20±3.76 to 17.97±2.75 mg/g, respectively, compared to those of DW green tea (Table 3). These results closely coincide with the NSA results of Table 1.

Colour analyses

The Hunter colour values of DW and DSW green teas are shown in Table 2. The L values of DW and DSW green tea extracts were 94.69±0.01 and 94.74±0.48, respectively. The redness (a value) and yellowness (b value) of DW and DSW green tea were -2.27±0.06 and -2.16±0.04 (a) and 13.38±0.13 and 13.64±0.03 (b), respectively. The overall colour change (ΔE) from DS to DSW green tea was 0.04, a difference that is difficult to detect with the naked eye.

Catechin analysis

Six types of catechins and caffeine in DW and DSW green tea were detected by using HPLC (Figure 1). As shown in Table 3, DSW increased the levels of catechins and caffeine. For example, EGC and EGCG in DW green tea vs. DSW green tea increased from 19.73 ± 0.43 mg/g to 21.82 ± 0.04 mg/g and from 13.20±3.76 mg/g to 17.97 ± 2.75 mg/g, respectively. The other epicatechins (EC and EGC) found in green tea were higher in DSW green tea than in DW green tea. DSW increased the total amount of epicatechins in green tea (48.66 mg/g) by more than 20% when compared to that of DW green tea (40.38 mg/g). The content of caffeine, a plant alkaloid having stimu-latory effects, was also affected by DSW. In DSW green tea, caffeine content increased to 15.42±0.02 mg/g from 13.14±0.34 mg/g in DW green tea. As shown in Figure 1 and Table 3, DSW significantly increased the EGC and EGCG contents of green tea. EGC and EGCG are reported to be the most important flavanols in green tea (Crespy and Williamson, 2004). Because the gallyl and galloyl moieties of GCs possess 3 hydroxyl groups and easily form radicals during oxidation, they exhibit the high hydrogen-donating ability of antioxidants. Increased EGC and EGCG may increase DPPH radical scavenging activity and reducing power. Many plant polyphenols, such as flavonoids, tannins, coumarins, curcuminoids, xanthones, phenolics, and terpenoids, exist either in bound form with high molecular weight compounds, or as parts of repeating subunits of high molecular weight polymers (Karamali and Teunisvan, 2001).

Gulati et al. (2003) reported that total phenol and catechin of green tea were increased by microwave treatment during manufacture, and they suggested that the application of microwave energy prevented the binding of polyphenols and catechins to the leaf matrix, which could increase the catechin content in green tea. Far-infrared irradiation during manufacturing green tea also released and increased the amount of catechins and caffeine with similar way to microwave irradiation (Kim et al., 2006). These results also indicated that DSW could liberate catechins and caffeine. The minerals in DSW may contribute to the increased levels of catechins and caffeine; however, the mechanism by which DSW exerts these effects remains unknown.

Conclusion

DSW significantly increases the antioxidant activity, NSA, and catechin and caffeine levels in green tea. These results suggest that the use of DSW in the preparation of green tea could enhance the health-promoting effects of green tea.

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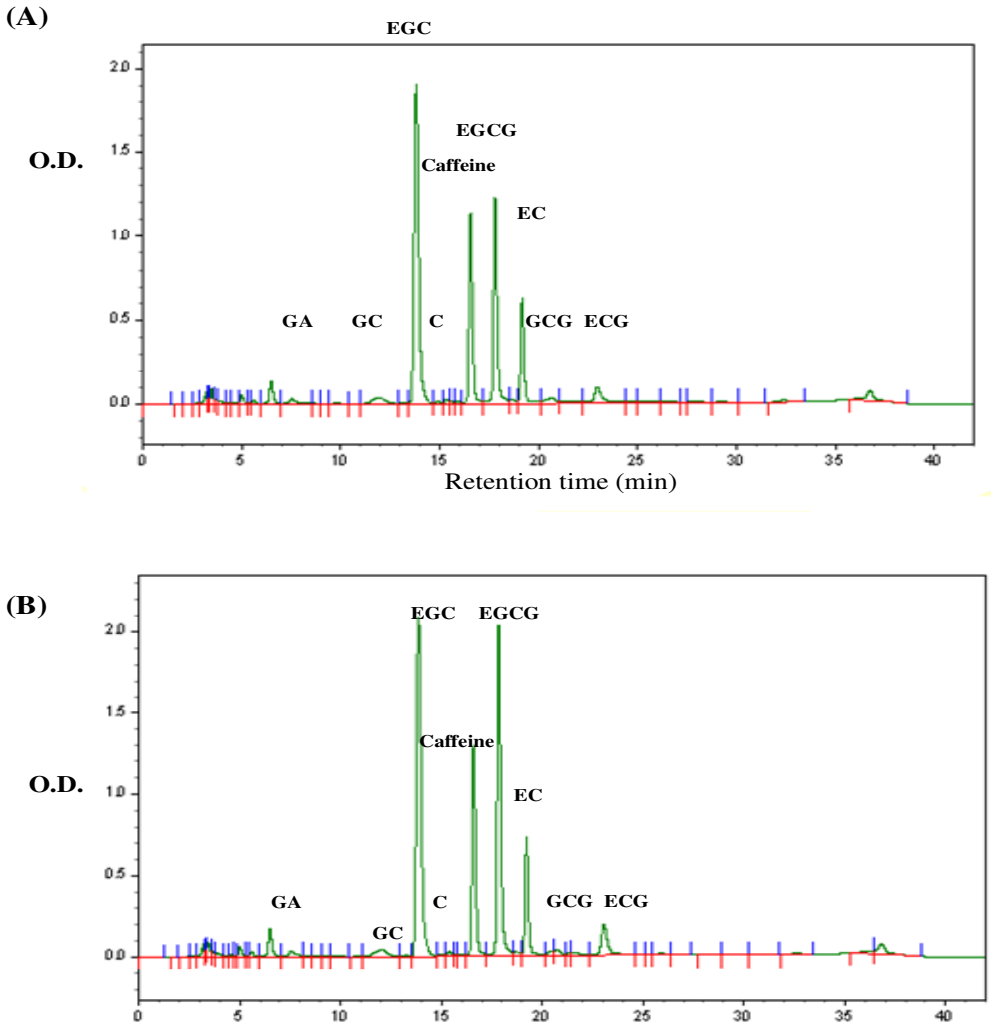

Figure 1. Typical HPLC chromatography of (A) DW green tea and (B) DSW green tea.

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