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Persimmon leaf and seed powders could enhance nutritional value and acceptance of green tea

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Acceptance of green tea, a health promoting functional food material, could be increased if its flavor is improved. Persimmon seeds and leaves are likely potential options for green tea improvement since they contain a large number of volatile flavor compounds. In the present study, amino acids, volatile flavor compounds, and antioxidant potentials of persimmon tea (PT) prepared with green tea were investigated. PT contained 8 essential amino acids, most of which are associated with learning ability and memory as well as stroke and neurodegenerative diseases prevention. Total free amino acid was high in persimmon seed tea (273.37-300.61 µg/mL) as compared to persimmon leaf tea (179.75-198.44 µg/mL). Total phenol content of persimmon leaf tea ranged from 333.07 to 348.87 GAE µg/g and that of seed tea from 324.63 to 356.73 GAE µg/g. Some of the volatile flavor compounds such as 2-undecanone; furfural; 5-methyl furfural; benzoic acid, methyl ester; benzaldehyde, 4-methyl; and phenol, 2,4-bis (1,1-dimethylethyl) were found in persimmon leaf and seed tea. The results of the present study suggest that persimmon leaf and seed could offer good options to enhance the preference of green tea along with its nutritional value.

Key words: Acceptance, green tea, nutritional value, persimmon leaf, persimmon seed.

INTRODUCTION

Persimmon fruit contains different nutrients and phytochemicals which significantly contribute to its taste, color, nutritive, and medicinal values (Celik and Ercisli, 2007; Del Bubba et al., 2009). Not only the fruits but also other parts of persimmon like leaves and seeds are found to contain different phytochemicals having nutritive and medicinal values. Persimmon seeds possess high antioxidant potential and also contain high level of unsaturated fatty acids (Jang et al., 2010). Persimmon leaf extract showed therapeutic potential to alleviate the severity of radiation-induced liver injury, hyperglycemia, hypoinsulinemia, and dyslipidemia in rats (Ashry et al.,

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2016). Results of Huang et al*.* (2016) indicated that persimmon leaves contained a potent protective effect on cognitive deficits induced by amyloid beta in rats.

Applications of leaves and seeds of persimmon in preparing different food and beverage items have been practiced. Lim and Lee (2016) prepared persimmon leaf powder (PLP) supplemented cookies and found to improve them with the functional properties of PLP, without compromising on consumer acceptance.

Hirayama et al. (2016) suggested that intake of persimmon leaf extract would be useful for lowering blood pressure in subjects with high normal blood pressure and stage I hypertension and have no safety concern for long-term intake.

Very little information exists about the volatile profile of seed and leaf of Korean persimmon cultivars. On the other hand, the possible use of seed and leaf of Korean persimmon cultivars in preparing tea has not been studied. Consumption of tea has been associated with reduced incidence of chronic diseases, like cancer (Butt and Sultan, 2009) and cardiovascular diseases (Stangl et al., 2007). Green tea may have even more benefits due to higher levels of bioactive compounds than in black tea (Wang et al., 2011). However, green tea is less preferred because of its flavor as compared to fermented black and semi-fermented 'Oolong teas' (Han et al., 2016). Fruit flavor is resulted from a combination of taste and aroma (Klee, 2010). The taste mainly depends on sugars and organic acids, whereas the aroma on a large number of volatile organic compounds (Xi et al., 2016). As persimmon contains a large number of volatile compounds the objective of this study was to investigate the potential use of persimmon leaf and seed as a supplement of green tea. The volatile compounds present in the seed and leaf tea of two Korean persimmon cultivars of astringent type were investigated. Amino acid composition as well as antioxidant potential of the tea samples was also described.

MATERIALS AND METHODS

Chemicals and materials

Folin-Ciocalteu phenol reagent and 2,2-diphenylpicrylhydrazyl radical (DPPH) were procured from Sigma-Aldrich (St. Louis, MO, USA). All other reagents were of analytical grade. Leaves and seeds; grown at Sangju Persimmon Experiment Station, Gyeonsangbuk-do, Korea; were obtained from the ready-to-eat maturity stage (November, 2015) of persimmon (*Diospyris kaki* Thunb. cv. Sangjudungsi and Sanggamdungsi). Green tea was obtained from a local store in Daegu, Korea in November of 2015.

Preparation of persimmon tea samples

Intact fresh leaves were harvested from the standing persimmon trees, kept into airtight plastic bags, and transported to the laboratory within 6 h of harvest. The leaves were washed with tap water, surface dried at room temperature, and freeze dried to make leaf powder. Freeze dried leaves were ground into powder using a ceramic ball mill (SW-BM117, Samwoo Engineering, Seoul, Korea) and strained using a series of US standard testing sieves (60 mesh) stacked in the portable sieve shaker (M-11630, Tyler Co., Salibury, NC, USA) for 10 min. The powder was collected, packed in zipper plastic bags, and stored at 4°C until analysis.

Seeds obtained from the healthy ready-to-eat stage persimmon fruits were washed with tap water to remove any adhering pulp and kept for hot air oven drying (60°C for 72 h). Dried seeds were ground using a roller mill (K-150, Hanil Co, Seoul, Korea) and seed powder were prepared as leaf powder.

Although persimmon contains a large number of volatile compounds, a commercial green tea (CGT) was added to improve the flavor of persimmon leaf and seed tea. Persimmon seed tea (PST) was prepared by mixing equal volume of seed powder and CGT and persimmon leaf tea (PLT) by mixing equal volume of seed powder and CGT. Two grams of persimmon seed or leaf powder were mixed with 2 g of CGT and the mixture (4 g) was extracted with 100 mL of boiling distilled water for 2 min. The extracts were filtered through 0.2 µm syringe filter (Waters, Milford, MA, USA) for further analyses. Persimmon tea samples of were named as SJLT, Sangjudungsi persimmon leaf tea; SJST, Sangjudungsi persimmon seed tea; SGLT, Sanggamdungsi persimmon leaf tea; and SGST, Sanggamdungsi persimmon seed tea.

Determination of free amino acid content of persimmon tea

Free amino acid composition was analyzed following the procedure of Je et al. (2005) with some modifications. Persimmon seed or leaf tea samples (3 g) were homogenized at 12000 rpm twice for 2 min with 20 mL of ice-cold 6% (v/v) perchloric acid in an ice bath using a homogenizer (HMF-985, Hanil). The homogenized sample was then incubated for 30 min in ice and centrifuged at 2000×*g* for 15 min. The residue was reextracted as described previously. The supernatants were combined and filtered through a Whatman No. 41 filter paper. The pH of the filtrate was adjusted to 7 using a 33% (w/v) KOH solution and centrifuged at 2000×*g* for 10 min to remove precipitate of potassium perclorate. The pH of the supernatant was adjusted to 2.2 with a 10 M HCl solution and then diluted to 50 mL with distilled water. Two milliliters of the extract was transferred into a clean tube and 1 mL of lithium citrate buffer (pH 2.2) was added to it. Samples were then analyzed using an automatic amino acid analyzer (Biochrom 20, Biochrom Ltd, Cambridge, UK).

Solid phase microextraction (SPME) procedures

SPME is a powerful sample preparation tool prior to mass spectrometric analysis. SPME parameters were optimized for extraction and desorption for persimmon tea volatiles. Ten milliliters of persimmon tea was added to a 40 mL glass vial containing a small Teflon-coated stirring bar with a screw top and Teflon-lined septum. After the equilibration time of 20 min, volatiles from the persimmon tea headspace were extracted for 30 min at 40°C using a 100 mm 50/30 µm DVB/Carboxen/PDMS SPME fiber (Supelco, Bellefonte, PA, USA). Before each exposure, the fiber was cleaned in a 260°C injection port for 5 min.

GC-MS

Gas chromatography – mass spectrometry, GC-MS (Clarus 500 quadruple, Perkin/Elmer, Shelton, CT, USA) analyses were equipped with a software (Turbo Mass, Perkin/Elmer). Conditions for GC-MS were as follows: Helium was used as the carrier gas with a constant flow mode of 2 mL/min. The source was kept at 200°C, and the transfer line and injector were kept at 220°C. Compounds were separated on a 60 m, 0.25 mm i.d., 0.5 µm DB-

Wax column (J&W Scientific, Folsom, CA, USA). The mass spectrometer was operated in the total ion chromatogram at 70 eV. Data were collected from 40 to 300 m/z. Mass spectra matches were made by comparison of NIST 2002 standard spectra (NIST, Gaithersburg, MD, USA).

Identification of volatile compounds

Initial identifications were based upon the matches made from spectra in the NIST (National Institute of Standards and Technology) library, aroma descriptors, and linear retention index matches from literature or from standards. The final confirmation was based upon the combined matching of retention indices (LRI values), full scan mass spectra values, and aroma descriptions from standards with those observed in the sample.

Determination of total phenol content of persimmon tea

The total phenol contents of persimmon seed tea were estimated according to the Folin-Ciocalteau method (Singleton et al., 1999). Fifty microliters of persimmon tea extract sample was mixed to 250 µL of Folin-Ciocalteau. After 1 min, 750 µL of aqueous solution of Na_2CO_3 (20%) was added, and the volume was made up to 5 mL using distilled water. The mixture was kept for 2 h at room temperature under dark condition and the absorbance value was measured at 760 nm using a spectrophotometer (Multiskan GO, Thermo Fisher Scientific Oy, Vantaa, Finland). Gallic acid was used to plot the standard calibration curve. The total phenol contents were determined as gallic acid equivalents (µg GAE/mL of sample), and values are reported as mean values of triplicate analyses.

DPPH radical scavenging activity

Antioxidant activity was measured with DPPH radical scavenging method according to Cheung et al. (2003) with modification. Eight hundred microliters of 0.2 mM DPPH ethanol solution was mixed with 0.2 mL of persimmon tea samples and kept for 30 min at room temperature under dark condition. The absorbance value was measured at 520 nm using a spectrophotometer (Multiskan GO, Thermo Fisher Scientific Oy).

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using SAS 9.4. Differences between means at $p<0.05$ were identified using Tukey test. Average values are presented as mean±standard deviation (SD).

RESULTS AND DISCUSSION

Free amino acid composition of persimmon tea

At least 19 free amino acids were found in all 4 tea samples; SJLT, SJST, SGLT, and SGST (Table 1). Total free amino acids content was higher in the seed tea samples than in leaf tea samples. Out of 19 free amino acids detected in all the samples, 6 were essential ones. Sixteen amino acids were not detected in either sample whereas L-citrulline and L-α-amino-n-butylric acid were detected only in seed tea samples of both cultivars and proline was found only in SGLT. Total free amino acids were highest in SJST (300.61 µg/mL) followed by SGST (273.37), SJLT (198.44), and SGLT (179.75). L-Aspartic acid (73.29-122.32 µg/mL) and L-Sarcosine (83.19-139.68) were the most abundant free amino acids found in the tea samples.

Amino acids γ-amino-n-butyric acid (GABA) and glycine were found in all the tea samples. These two amino acids are associated with the learning and memory, stroke and neurodegenerative diseases; mediate signals between neurons that inhibit neutral amino acids and thus relieve anxiety, sedation, anticonvulsant, and muscle relaxation functions (Mody et al., 1994; Oh and Oh, 2004). GABA rich foods are considered as brain food because of its bioactive capabilities to enhance blood cholesterol and triglyceride blood pressure suppression, improved cerebral blood flow, antioxidant, diuretic, insomnia, depression and anxiety stabilizing effect on nerves, and pain (Dhakal et al., 2012). The amount of amino acids is one of the key factors in determining the nutritional qualities of food materials (Basarova and Janousek, 2000). The variation in the amino acid content in persimmon tea samples might be due to variation in cultivar since pomological properties of fruit are strongly affected cultivar variation (Mratinic et al., 2011). Moreover, Park et al. (2003) found changes in organic nutrients of persimmon leaves by differing in the time of abscission.

Volatile flavor compounds of persimmon leaf and seed tea samples

Volatile flavor compounds identified in leaf tea are shown in Table 2 and those in seed tea are in Table 3. Nine volatile flavor compounds were detected in SJLT whereas SGLT contained 13 compounds. Volatile compounds, 1-octen-3-yl acetate; 3-heptafluorobutyroxypentadecane 3-trifluoroacetoxytridecane; 2-pentadecanone, 6,10,14-trimethyl; 3-heptanol; 2-hexanol, 2,5-dimethyl; and octane, 4-methyl were not detected in SJLT whereas hexadecamethyl; nonanoic acid; and benzoic acid were not detected in SGLT. Relatively low number of volatile flavor compounds was detected in seed tea of ether cultivar as compared to leaf tea (Tables 2 and 3). There were seven volatile flavor compounds identified in SJST whereas nine compounds were detected in SGST. The only compound that was detected in SJST but not in SGST was 3-(4-tertiobutylphenyl)-propanal. Compounds 5-methyl furfural; 2-furancarboxylic acid, methyl ester; and 1,4,7,10,13,16-hexaoxacyclooctadecane were detected in SGST but not in SJST.

Wang et al. (2012) identified 50 volatile and aroma-impact compounds from persimmon fruits of Triumph cultivar. Thirty-eight volatile compounds were detected using steam distillation from leaves of 3 persimmon cultivars; Fuyugaki, Fujigaki, and Jitsuseisibugaki (Kameoka et al., 1989). The difference in the volatile

Table 1. Free amino acid composition (μg/mL) of persimmon leaf and seed tea samples.

Amino acid	Sample[1)]			
	SJLT	SGLT	SJST	SGST
O-Phospho-L-serine	ND[2)]	ND	ND	ND
Taurine	ND	ND	ND	ND
O-Phospho ethanol amine	ND	ND	ND	ND
Urea	ND	ND	ND	ND
L-Aspartic acid	73.29[3)]	81.34	122.32	114.84
L-Threonine	0.89	0.92	1.38	1.34
L-Serine	2.28	2.40	3.61	3.49
L-Glutamic acid	8.61	9.43	14.81	13.66
L-Sarcosine	83.19	87.89	139.68	122.38
L-α-Aminoadipic acid	ND	ND	ND	ND
Glycine	0.71	0.73	0.73	0.79
L-Alanine	1.61	1.65	2.41	2.25
L-Citrulline	ND	ND	1.16	1.35
L-α-Amino-n-butylric acid	ND	ND	0.28	0.43
L-Valine	0.92	1.02	1.17	1.12
L-Cystine	2.79	3.23	4.95	4.29
L-Methionine	ND	ND	ND	ND
Cystathionine	ND	ND	ND	ND
L-Isoleucine	0.27	0.23	0.31	0.28
L-Leucine	0.67	0.63	0.74	0.71
L-Tyrosine	0.41	0.45	0.46	0.42
L-Phenylalanine	0.81	0.74	0.95	1.02
β-Alanine	0.05	0.05	0.08	0.06
D, L-β-Aminoisobutyric acid	ND	ND	ND	ND
γ-Amino-n-butyric acid	1.32	1.35	2.27	2.04
Ethanolamine	ND	ND	ND	ND
Ammonia	0.94	1.02	1.28	1.12
Hydroxylysine	ND	ND	ND	ND
L-Ornithine	ND	ND	ND	ND
L-Lysine	0.38	0.49	0.87	0.68
1-Methyl-L-histidine	0.37	0.42	0.59	0.59
L-Histidine	ND	ND	ND	ND
3-Methly-L-histidine	0.23	0.36	0.54	0.48
L-Anserine	ND	ND	ND	ND
L-Carnosine	ND	ND	ND	ND
L-Arginine	ND	ND	ND	ND
Hydroxy proline	ND	ND	ND	ND
Proline	ND	4.08	ND	ND
Total free amino acid	179.75	198.44	300.61	273.37

[1)]SJLT, Sangjudungsi cultivar's leaf tea; SGLT, Sanggamdungsi cultivar's leaf tea; SJST, Sangjudungsi cultivar's seed tea; SGST, Sanggamdungsi cultivar's seed tea. [2)]Not detected. [3)]Quoted values are average of duplicate experiments.

compound in the present study from those of previous ones might be due to variation in cultivar since pomological properties of fruit are strongly affected cultivar variation (Mratinic et al., 2011).

Incredible efforts have been made to comprehend the variations in the volatile constituents of green tea (Hara et al., 1995). Investigation on profiling the volatile compounds of green tea enhances the understanding of tea flavors specifically perceived by consumers (Schuh and Schieberle, 2006; Baba and Kumazawa, 2014). Studies on compelling odorants of green tea are crucial to the characteristic aroma of the tea (Kumazawa and

Table 2. Volatile flavor compounds identified in Sangjudungsi leaf tea (SJLT) and Sanggamdungsi leaf tea (SGLT).

RT[1)] (min)	Compounds	Peak area (%)	
		SJLT	SGLT
7.942	1-Octen-3-yl acetate	ND[2)]	1.1
8.039	2-Undecanone	4.2	6.51
8.436	3-Heptafluorobutyroxypentadecane 3-Trifluoroacetoxytridecane	ND	0.64
9.397	2-Pentadecanone, 6,10,14-trimethyl-	ND	0.8
11.88	Nonanol, trimethyl-	2.33	5.13
12.42	3-Heptanol	ND	2.06
12.76	2-Hexanol, 2,5-dimethyl-	ND	1.56
13.71	Octane, 4-methyl-	ND	1.8
19.79	Furfural	25.1	12.64
25.56	5-Methyl furfural	4.56	2.08
27.79	Benzoic acid, methyl ester	5.91	4.97
29.06	Benzaldehyde, 4-methyl-	15.9	12.1
55.51	Nonanoic acid	4.3	ND
62.05	Phenol, 2,4-bis(1,1-dimethylethyl)-	19	11.36
66.31	Benzoic acid	6.86	ND

[1)]Retention time. [2)]Not detected.

Table 3. Volatile flavor compounds identified in Sangjudungsi seed tea (SJST) and Sanggamdungsi seed tea (SGST).

RT[1)] (min)	Compounds	Peak area (%)	
		SJST	SGST
8.039	2-Undecanone	5.86	5.51
11.87	Acrylic acid, 2-acetamido-	3.09	2.01
17.83	3-(4-tertiobutylphenyl)-propanal	4.64	ND
19.80	Furfural	6.58	11.57
25.56	5-Methyl furfural	ND[2)]	2.00
25.86	2-Furancarboxylic acid, methyl ester	ND	4.05
27.79	Benzoic acid, methyl ester	9.58	6.78
29.06	Benzaldehyde, 4-methyl-	20.09	17.71
62.05	Phenol, 2,4-bis(1,1-dimethylethyl)-	30.15	22.40
70.67	1,4,7,10,13,16-Hexaoxacyclooctadecane	ND	3.66

[1)]Retention time. [2)]Not detected.

Masuda, 1999; Baba and Kumazawa, 2014). In such circumstances, use of persimmon leaves or seeds could enhance the preference of green tea by enriching its flavor as it is less preferred because of its flavor as compared to fermented black and semi-fermented Oolong teas (Han et al., 2016). Although the effect of persimmon leaf on sensory properties of green tea is not reported, addition of chopped persimmon leaves gave significantly high scores for color, flavor, texture and overall preference to kimchi, a Korean traditional famous food (Park et al., 2010).

Antioxidant activity of persimmon seed tea

Antioxidant potential of persimmon tea was measured by α-diphenyl-β-picrylhydrazyl (DPPH) free radical scavenging activity and content of phenols (Table 4). The DPPH radical-scavenging potential of SGST was significantly (p<0.05) high as compared to the other 3 tea samples. Total phenol content of SGLT (348.87 µg/g) and SJST (356.73 µg/g) were significantly (p<0.05) higher than those of SJLT (333.07 µg/g) and SGST (324.63 µg/g).

Since DPPH method evaluates the concentration of radical-scavenging materials actively by a chain-breaking mechanism, it is considered as one of the most effective methods to determine the antioxidant activity of plant extracts (Niki, 1987). The results of DPPH study in this study showed that the leaf and seed tea of persimmon are good free radical scavengers. Results of Jang et al. (2010) and Akter et al. (2010) also showed that

Table 4. DPPH radical scavenging activities and total phenol contents of persimmon leaf and seed tea samples.

Sample[1)]	DPPH (% Inhibition)	Total phenol content (GAE[3)] μg/g of sample)
SJLT	84.89±0.02[b2)]	333.07±3.12[b]
SGLT	84.51±0.87[b]	348.87±6.00[a]
SJST	84.26±0.31[b]	356.73±2.11[a]
SGST	87.22±0.71[a]	324.63±0.88[b]

[1)]SJLT, Sangjudungsi cultivar's leaf tea; SGLT, Sanggamdungsi cultivar's leaf tea; SJST, Sangjudungsi cultivar's seed tea; SGST, Sanggamdungsi cultivar's seed tea..[2)]Values are the mean±SD of triplicate experiments. Different superscripts in the same column followed by the values are significantly different at $p<0.05$.[3)]Gallic acid equivalents.

persimmon seeds possess high antioxidant potential. High antioxidant potential of persimmon leaves has also been reported in previous studies (Martínez-Las Heras et al., 2014; Lim and Lee, 2016). The results of the present study showed that persimmon tea samples contain good antioxidant potentials. Difference in antioxidant properties of persimmon tea samples might be due to the difference in the amount and form of tannin, the main phenolic compound of persimmon (Jang et al., 2011). Kim et al. (2015) mentioned that persimmons contain a specific flavonoid, cyanidin which was not found in green tea. Astragalin (kaempferol-3-O-glucoside), a flavonoid having anti-tumor, anti-inflammatory, and antioxidant activity have been found from leaves of persimmon and green tea seeds (Burmistrova et al., 2011; Kim and Kim, 2011). This report implies that addition of persimmon leaf and or seed powder to green tea further enhances its antioxidant potential. In addition, tannins from persimmon were found to show more anti-viral effects against a broad range of viruses than those derived from green tea (Ueda et al., 2013).

In conclusion, persimmon tea samples contained 8 out of 9 essential amino acids needed for human. In addition, amino acids like γ-aminobutyric acid (GABA) and glycine; which are associated with the learning and memory as well as stroke and neurodegenerative diseases were found in all the tea samples. A considerable number of volatile compounds are found in persimmon tea samples. Green tea, which is considered as health promoting functional food material, is less preferred because of its flavor as compared to fermented black and semi-fermented. Use of persimmon leaves and or seeds showed potentiality of enhancing the preference of green tea by enriching its flavor. From the antioxidant perspective, adding persimmon leaf and or seed to the green tea is to enhance its antioxidant potentiality. The results of the present study showed that persimmon leaf and seed tea offer a good choice to enhance the preference of green tea as well as enrich its antioxidant potentials.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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