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Persimmon (*Diospyros kaki* Thunb.) seed: a potential nutritional source with antioxidant and pharmaceutical activity

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Abstract

Pulp is a major part of persimmon fruit, for which it is widely cultivated in different parts of the world. Persimmon seeds are generally discarded as waste. The objective of the present work was to investigate the nutritional, antioxidative, and pharmacological properties of the seeds of three persimmon cultivars namely Jinyeong (JYS), Yangyangdongchulsi (YYS), and Hamanmulgam (HAS). JYS (9417.87 mg.kg^{-1}) contained the highest concentration of total minerals, followed by HAS and YYS. The concentration of total organic acids was also the highest in JYS (5362.43 mg.kg^{-1}), while the lowest in HAS (4411.1 mg.kg^{-1}). Similarly, JYS and YYS contained the highest and lowest concentrations of free sugars, respectively. On the other hand, the total free amino acid contents were the highest in YYS (336.34 mg.100 g^{-1}) among the cultivars. The persimmon seeds also have good potential for scavenging free radicals and inhibiting acetylcholinesterase activity. The seeds could also be considered to be a source of a therapeutic agent against Alzheimer's disease, as the seed extract showed promising anti-acetylcholinesterase activity. The overall results of the present work provide an insight into persimmon seeds, a by-product of persimmon fruit, as a potential product in the pharmaceutical and food industries.

Introduction

Fruit pulp is a major part of persimmon (*Diospyros kaki* Thunb.), for which it is cultivated in many parts of the world, especially in Korea, China, and Japan. The fruits are consumed raw, or subjected to drying for preservation. Persimmon fruits are rich in nutrients and phytochemicals, which account for their high antioxidant, anti-infection, anti-inflammatory, and antihemorrhagic properties (Kim *et al.,* 2006). Persimmon seeds, which are generally discarded as by-products of persimmon also possess different antioxidants as do many other fruits' seeds such as rambutan, mango, tamarind, and berry (Maisuthisakul *et al.,* 2007). Persimmon seeds show a strong radical scavenging potential in vitro, and inhibit lipid peroxidation *in vivo* (Ahn *et al.,* 2002). Interestingly, seed and calyx extracts of persimmon have significantly higher antioxidant activity and phenolic content than the fruit peel and flesh extracts, which results in a high protective effect against oxidative DNA damage (Jang *et al.,* 2010). The seeds have more than ten times higher ferric reducing antioxidant potential than that of the pulp (Guo *et al.,* 2003).

Reactive oxygen species (ROSs) are generated in living organisms as a result of normal cellular metabolism, and are not harmful at moderate concentrations; however, at high concentrations, they produce adverse modifications to cell components such as lipids, proteins, and DNA (Marnett, 1999). An imbalance between an oxidant and antioxidant in favour of the former, known as oxidative stress, is responsible for many health problems including cancers, neurological disorders (Sayre *et al.,* 2001), and hypertension (Kerr *et al.,* 1999). Antioxidants are of significant importance in the prevention and treatment of several cellular degenerations by blocking the initiation or propagation of oxidising chain reactions and consequently inhibiting or delaying the oxidation process (Behera *et al.,* 2006). The antioxidants interfere with the production and the activation of free radicals, which protect the human body from chronic diseases. Because the use of synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene, and propyl gallate has

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been questioned, and a negative perception of taking such antioxidants has developed among consumers due to long-term safety concerns, the demand for natural antioxidants has increased (Yu *et al.,* 2002).

Acetylcholinesterase (AChE) is a vital enzyme in the nervous system that checks nerve impulses by proper hydrolysis of a neurotransmitter, acetylcholine. AChE inhibitors are of significant importance for controlling several neurological problems, and are accepted as the major drug for the treatment of Alzheimer's disease.

Although there are few reports on the antioxidant potential of persimmon seeds, little work has been done to investigate the nutritional and pharmacological properties of persimmon seeds of different cultivars. A collaborative research concluded that persimmon seeds have the potential to enhance the acceptance of green tea (Kim *et al.,* 2017). In a previous study, we investigated the biochemical constituents and antioxidant potential of persimmon seeds of four cultivars: Sangju Doongsi, Gyoungsan Bansi, Jinan Galgam, and Sanggam Doongsi (Bilal *et al.,* 2016). Previous studies (Ahn *et al.,* 2002; Jang *et al.,* 2010; Bilal *et al.,* 2016) have focused on the antioxidant potentials of persimmon seeds. The objective of the present work was therefore to further investigate the nutritional and antioxidant potentials of the seeds of three other persimmon cultivars.

Materials and methods

Seed materials

Persimmon seeds of three cultivars, namely Jinyeong (JYS), Yangyangdongchulsi (YYS), and Hamanmulgam (HAS) were collected from Persimmon Experiment Station, Sangju, Korea. The three cultivars are among the widely cultivated persimmon cultivars in Korea. At least ten fruits were harvested from ten plants of each cultivar at the ready-to-harvest stage. The seeds were collected from the fruits, thoroughly rinsed with tap water, and allowed to surface dry at room temperature. The seeds were freeze-dried, finely ground (HIL-G-501, Hanil Co., Seoul, Korea), passed through a 60-mesh filter, and stored at -20°C until further analyses.

Determination of mineral contents

A 0.5-g of sample powder was mixed with 15 mL of 65% nitric acid (HNO_3). The mixture was diluted with 50 mL of distilled water. An inductively coupled plasma (ICP) emission spectrometer (38 Plus, Jobin Yvon, Co., Palaiseau, France) was used to determine the essential mineral elements, and an atomic absorption spectrometer (AA-220FS, Varian Spectra, Victoria, Australia) was used to analyse the heavy metals following a method described earlier (Gonzálvez *et al.,* 2010). The concentration of mineral elements was determined after calibration of the instruments with the respective standard elements.

Determination of organic acid contents

The concentration of organic acids was measured using high-performance liquid chromatography (HPLC) following the procedure reported by Ergönül and Nergiz (2010) with some modifications. Ten grams of seed powder was extracted with 10 mL of a water/methanol solution (75:25, v/v) at room temperature, and centrifuged (1,850 *g*, 30 min). One millilitre of the sample extract was added to 9 mL of distilled water, kept overnight at room temperature, and filtered through a 0.22 μm syringe filter (Millipore, Billerica, MA, USA). Conditions for the HPLC were: detector (M996, Waters, Milford, MA, USA); refractive index detector (RI410, Waters); mobile phase, 0.005 $mol.L^{-1}$ H_2SO_4 in water; column (PL Hi-Plex H, 300 × 7.7 mm, Agilent Technologies, Seoul, Korea); column temperature, 65°C; flow rate, 0.6 $mL.min^{-1}$; and injection volume, 10 μL.

Determination of free sugar contents

The free sugar content was examined by following the method described by Génard and Souty (1996). Five grams of sample powder was mixed with 10 mL of distilled water, homogenised using a homogenizer (Ultra-Turrax T-25, IKA-Lobortechnik, Staufen, Germany), and followed by the addition of 20 mL of distilled water and centrifugation (16,000 *g*, 30 min). The supernatant was filtered through a Sep-Pak C_{18} cartridge (WAT023501, Waters) and a Millipore 0.45-syringe filter (PVDF, Whatman, Tokyo, Japan). The quantification of free sugars was performed by HPLC (Model 9300, Young Lin, Gyeonggi-do, Korea) under the following conditions: a column heater set at 85°C, sugar-pak (6.5 × 300 mm, Alltech, Atlanta, GA, USA), and a mobile phase of deionised H_2O delivered at a rate of 0.5 $mL.min^{-1}$. Glucose, fructose, sucrose, and sorbitol (Aldrich Chemical Co. Inc., Milwaukee, WI, USA) were used as the reference sugars for identification, and mannitol (Aldrich Chemical Co. Inc.) was used as an internal standard.

Determination of free amino acids

The free amino acids were determined following the method described by Spackman *et al.* (1958) with some modifications. One gram of sample powder was hydrolysed with 6 N HCl (10 mL) in a vacuum-sealed ampoule at 110°C for 24 h. The HCl was

removed from the hydrolysed sample using a rotary evaporator, and the remaining sample was diluted to a known volume (5 mL) with 0.2 M sodium citrate buffer (pH 2.2). The mixture was passed through a C_{18} Sep Pak (Waters Co. Milford, USA) cartridge and filtered through a 0.22 µm membrane filter (Millipore, Billerica). An automatic amino acid analyser (Biochrom-20, Pharmacia Biotech Co., Stockholm, Sweden) was used for determining the amino acid profile.

DPPH radical scavenging activity

The 1,1-diphenyl-2-pricrylhydrazyl (DPPH; Sigma Chemical Co., St. Louis, Mo, USA) radical scavenging activity was measured following the method described by Sanna *et al.* (2012) with some modifications. A 0.5-mM solution of DPPH in methanol and a 0.05 M solution of acetate buffer (pH 5.5) were prepared. A 0.1-mL aliquot (at a concentration of 1 $mg.mL^{-1}$ w/v) of the extract solution was added to a mixture of 2 mL acetate buffer, 1.9 mL methanol, and 1 mL DPPH (0.5 mM) solution. The blank sample contained a mixture of 2 mL acetate buffer and 1.9 mL methanol, whereas the control sample contained a mixture of 2 mL acetate buffer, 1 mL DPPH, and 2 mL methanol. The mixture was thoroughly mixed using a vortexer (KMC-1300V, Vision Scientific Co. Ltd., Bucheon, Korea) immediately after the addition of DPPH, and then allowed to stand at room temperature in the dark for 30 min. The absorbance value was measured at 517 nm using a spectrophotometer (UV-1700, Shimadzu, Kyoto, Japan). Vitamin C (25 ppm) was used as a positive control to compare the DPPH free radical scavenging potential of persimmon seeds.

Superoxide anion scavenging activity

The superoxide anion scavenging activity was determined by measuring the inhibition of the auto-oxidation of pyrogallol (Li, 2012). Three hundred microliters of the sample solution and 2.61 mL of 50 mM phosphate buffer (pH 8.24) were added to 90 µL freshly prepared 3 mM pyrogallol solution (dissolved in 10 mM hydrochloric acid). The inhibition rate of pyrogallol autoxidation was measured at 325 nm using a spectrophotometer (UV-1700, Shimadzu). The absorbance of each extract was recorded for 10 min every minute and the difference in the absorbance was calculated from the absorbance values at 10 min and those at the beginning. The superoxide anion scavenging activity of the persimmon seed samples was compared using vitamin C (25 ppm) as a positive control.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was measured following a previous method (Chung *et al.,* 1997). Hydroxyl radicals were generated by Fenton reaction in the presence of $FeSO_4 \cdot 7H_2O$ (Acros Organics, Pittsburgh, PA, USA), that is, 10 mM of EDTA (Sigma Chemical Co., St. Louis, Mo, USA) and 10 mM 2-deoxyribose (Sigma Chemical Co., St. Louis, Mo, USA) were mixed with 0.2 mL of the sample extract, and the final volume (1.8 mL) was made up with 0.1 M phosphate buffer (pH 7.4). Then, 0.2 mL of 10 mM H_2O_2 (Merck Group, Darmstadt, Germany) was added to the reaction mixture and incubated at 37°C for 4 h. After the incubation, 1 mL of each 2.8% TCA (Sigma Chemical Co., St. Louis, Mo, USA) and 1% TBA (Sigma Chemical Co., St. Louis, Mo, USA) was added to the mixture. The mixture was placed in a boiling water bath for 10 min, allowed to cool to room temperature, and then the absorbance was measured at 532 nm using a spectrophotometer (UV-1700, Shimadzu). Vitamin C (25 ppm) was used as a positive control to compare the hydroxyl radical scavenging potential of the seed samples.

Acetylcholinesterase activity assay

The assay for measuring AChE activity was performed following a modified Ellman's spectrophotometric method (Ellman *et al.,* 1961), in which the AChE was obtained from *Electrophorus electricus*. Four hundred microliters of 50 mM tris buffer (pH 8), 50 µL of sample extract, and 25 µL of an enzyme solution containing 0.26 $U.mL^{-1}$ were mixed and incubated at room temperature for 10 min. Afterward, 75 µL of 15 mM acetylthiocholine iodide (AChI) and 475 µL of 3 mM 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) were added to the mixture. The method was based on measuring the concentration of the yellow anion of DTNB formed by performing an absorbance reading at 412 nm after 5 min of incubation at room temperature. Tacrine was used as a positive control.

Statistical analysis

The data were analysed using analysis of variance (ANOVA) and the differences among sample means were determined by Tukey's test using SAS9.4 (SAS Institute, Inc., Cary, NC, USA). *p*-values of less than 0.05 ($p < 0.05$) were considered to be significantly different. The average values of the three replications were reported unless otherwise mentioned.

Results and discussion

Nutritional properties of persimmon seed

The nutritional properties of the persimmon seeds were evaluated based on the mineral, organic acid, free sugar, and free amino acid contents.

The mineral content of persimmon seeds of different cultivars significantly varied ($p < 0.05$) (Table 1). K was the most abundant (the highest were 6,100.21 mg.kg^{-1}, and the lowest were 5,100.17 mg.kg^{-1} in JYS and YYS, respectively) mineral element in the persimmon seeds. Ca was the second-most abundant mineral detected, with the highest concentration in HAS and the lowest concentration in YYS. YYS (1,240.27 mg.kg^{-1}) had the highest and JYS (1,180.31 mg.kg^{-1}) had the lowest Mg content among the three cultivars. The highest concentrations of Fe (351.21 mg.kg^{-1}) and Mn (129.50 mg.kg^{-1}) were found in JYS, whereas HAS contained the lowest concentration (31.91 and 15.17 mg.kg^{-1}), respectively. Zn was detected only in JYS (81.12 mg.kg^{-1}). None of the three cultivars showed any trace of heavy metals such as As, Pb, Cd, or Hg. The total mineral content in JYS was higher than in the other two cultivars. The significant variation in mineral contents might be owing to the genotypic variation among cultivars, as pomological characteristics of fruits are strongly affected by the genotypes (Ganji Moghaddam *et al.,* 2013). To the best of our knowledge, this is the first report on the mineral content of persimmon seeds. The results of the present work show that persimmon seeds could be a potential source of dietary mineral elements without any detectable concentration of four heavy metals. Some of the mineral elements such as Zn and Mn that were found in the present work were up to ten times higher than that found in persimmon fruits (Kim *et al.,* 2016). Similarly, the concentrations of K, Mg, Ca, Na, Fe, Zn, and Mn found in the present work were higher than those detected in cashew nuts (Soares *et al.,* 2012). The high potassium content in the persimmon seeds revealed their potential to be used as food additives because potassium intake has been inversely related to abdominal obesity and fasting hyperglycaemia in adults (Shin *et al.,* 2013).

Out of the six organic acids analysed, lactic acid was detected only in YYS (1,500.1 mg.kg-*1*), whereas tartaric acid was found in the other two cultivars. The most abundant organic acid was acetic acid, with the highest concentrations in JYS (5,201.7 mg.kg^{-1}) and the lowest in YSS (2,011.2 mg.kg^{-1}). YYS contained a significantly high concentration of oxalic (121.2 mg.kg^{-1}) and malic acids (341.4 mg.kg^{-1}) (Table 2). The total organic acids content of JYS (5,362.4 mg.kg^{-1}) was the highest, followed by HAS (4,411.1 mg.kg^{-1}) and YYS (3,975.3 mg.kg^{-1}). The total organic acid content of JYS and HAS was higher than that found in a previous study (Bilal *et al.,* 2016). Among the organic acids, the concentrations of tartaric and malic acids found in the present work were up to seven and ten times higher, respectively, than those found in grape seeds (Lamikanra *et al.,* 1995). However, the total organic acid content of persimmon seeds detected in the present study was approximately three times lower than that in persimmon pulp (Kim *et al.,* 2016). Organic acids

Table 1. Mineral content (mg.kg^{-1} on a dry basis) of seeds of three Korean persimmon cultivars.

Mineral element	Cultivar		
	JYS	YYS	HAS
K	6,100.21 ± 6.71^{a}	5,100.17 ± 9.22^{c}	5,666.25 ± 10.00^{b}
Mg	1,180.31 ± 7.92^{c}	1,240.27 ± 6.23^{a}	1,222.66 ± 7.01^{b}
Ca	1,400.27 ± 9.21^{b}	1,209.22 ± 9.21^{c}	1,446.55 ± 7.77^{a}
Na	175.25 ± 1.51^{c}	199.21 ± 3.12^{a}	191.31 ± 2.00^{b}
Fe	351.21 ± 3.92^{a}	61.00 ± 2.15^{b}	31.91 ± 1.99^{c}
Zn	81.12 ± 1.51	ND	ND
Mn	129.50 ± 6.36^{a}	54.88 ± 1.00^{b}	15.17 ± 2.01^{c}
As	ND	ND	ND
Pb	ND	ND	ND
Cd	ND	ND	ND
Hg	ND	ND	ND
Total	9,417.87	7,864.75	8,573.85

JYS = Jinyeong; YYS = Yangyangdongchulsi; and HAS = Hamanmulgam. Values are expressed as mean ± SD. Different superscripts within a row indicate significant differences ($p < 0.05$). ND = not detected.

and sugars contribute to the quality and nutritional value of foods. The organic acids also have a protective role against various diseases as they have good antioxidant potentials (Valentão *et al.,* 2005). The organic acids content of persimmon seed makes them a potential source of natural antioxidants. In addition, because of the high acetic acid content in the persimmon seeds, they can be used as a food preservative, as short-chain organic acids such as acetic acid is considered appropriate for this purpose (Mari *et al.,* 2016). The health benefits of vinegar, such as blood glucose level control, lipid metabolism regulation, and weight loss capabilities are mainly owing to acetic acid (Chen *et al.,* 2016).

Table 2. Organic acid content (mg.kg^{-1} on a dry basis) of seeds of three Korean persimmon cultivars.

Organic acid	Cultivar		
	JYS	YYS	HAS
Oxalic acid	83.7 ± 3.1^{b}	121.2 ± 7.1^{a}	73.2 ± 0.7^{c}
Tartaric acid	35.5 ± 2.1^{b}	ND	83.2 ± 1.2^{a}
Malic acid	34.5 ± 7.2^{c}	341.4 ± 9.2^{a}	192.3 ± 2.0^{b}
Lactic acid	ND	1,500.1 ± 8.9	ND
Acetic acid	5,201.7 ± 6.2^{a}	2,011.2 ± 5.2^{c}	4,050.4 ± 9.2^{b}
Fumaric acid	7.0 ± 0.1^{b}	1.4 ± 0.3^{c}	12.0 ± 1.2^{a}
Total	5,362.4	3,975.3	4,411.1

JYS = Jinyeong; YYS = Yangyangdongchulsi; and HAS = Hamanmulgam. Values are expressed as mean ± SD. Different superscripts within a row indicate significant differences ($p < 0.05$). ND = not detected.

Free sugars such as fructose, glucose, and sucrose were detected in the persimmon seeds of the three cultivars; however, xylose, arabinose, mannose, maltose, and lactose were not (Table 3). The highest concentration of sucrose was detected in JYS (5,000.71 mg.100 g^{-1}) followed by YYS (2,200.31 mg.100 g^{-1}), whereas no detectable trace of sucrose was detected in HAS. Glucose was the second-most abundant free sugar, with the highest concentration found in HAS (2,640.01 mg.100 g^{-1}) and the lowest in YYS (100.11 mg.100 g^{-1}). The share of fructose as a free sugar was the highest in HAS (1,360.35 mg.100 g^{-1}) and the lowest in YYS (100.31 mg.100 g^{-1}). Similar to the organic acids, the highest content for free sugar was found in JYS followed by HAS and YYS. The free sugars contribute to the energy source of diets, and a well-balanced energy supply is crucial for maintaining a healthy body weight and ensuring optimal nutrient intake (Elia and Cummings, 2007). To the best of our knowledge, this is the first report on the free sugar content of persimmon seeds. The concentration of fructose and glucose found in the present work was much lower, whereas the concentration of sucrose was higher than those found in the persimmon fruits (Kim *et al.,* 2016). The concentration of total free sugars found in the present work was approximately 13 (Song *et al.,* 2019) and three (Kim *et al.,* 2016) times lower than that in jujube and persimmon fruits, respectively.

A total of 39 free amino acids were analysed; however, 12 of them were not detected in any of the three seed samples (Table 4). Fifteen amino acids were found in all three cultivars. Among them, citrulline (147.77 mg.100 g *-1*) was the most abundant followed by homocysteine (142.39 mg.100 g $^{-1}$). The total free amino acid content was slightly higher in YYS (327.33 mg.100 g^{-1}) when compared with the other two cultivars. The concentration of total amino acids in JYS and YYS was in the range, whereas that of HAS was lower than that found in a previous study (Bilal *et al.,* 2016). The total free amino acid content of persimmon seeds detected in the present work was in the range of that in persimmon fruits (Kim *et al.,* 2016), but much lower than that in jujube fruits (Song *et al.,* 2019). The essential amino acid content was the highest in JYS (42.14 mg.100 g^{-1}) among the three cultivars. The ratio of essential to non-essential

Table 3. Free sugar content (mg.100 g^{-1} on a dry basis) of seeds of three Korean persimmon cultivars.

Free sugar	Cultivar		
	JYS	YYS	HAS
Arabinose	ND	ND	ND
Fructose	220.51 ± 5.12^{b}	100.31 ± 1.81^{c}	1,360.35 ± 12.11^{a}
Glucose	470.71 ± 7.21^{b}	100.11 ± 1.72^{c}	2,640.01 ± 12.2^{a}
Lactose	ND	ND	ND
Maltose	ND	ND	ND
Mannose	ND	ND	ND
Sucrose	5,000.71 ± 11.21^{a}	2,200.31 ± 8.93^{b}	ND
Xylose	ND	ND	ND
Total	5,691.93	2,400.73	4,000.36

JYS = Jinyeong; YYS = Yangyangdongchulsi; and HAS = Hamanmulgam. Values are expressed as mean ± SD. Different superscripts within a row indicate significant differences ($p < 0.05$). ND = not detected.

amino acids was the highest in JYS (0.63). Foods with a higher ratio of essential to non-essential amino acids are considered well-balanced for protein deposition (Reeds, 2000). The results showed that persimmon seeds contained a wide variety of amino acids. Amino acids are the building blocks of tissue proteins and are vital substrates for the synthesis of many low-molecular-weight substances such as polyamines, glutathione, creatine, carnitine, carnosine, thyroid hormones, serotonin, melanin, melatonin, and haem, which have significant physiological importance (Wu, 2009; Kong *et al.,* 2012).

Table 4. Free amino acid content (mg.100 g^{-1} on a dry basis) of seeds of three Korean persimmon cultivars.

Amino acid	Cultivar		
	JYS	YYS	HAS
Essential amino acid			
Histidine	ND	ND	4.30
Isoleucine	6.59	4.76	6.46
Leucine	7.79	4.47	4.14
Lysine	ND	ND	2.93
Methionine	ND	ND	ND
Phenylalanine	ND	ND	ND
Threonine	10.97	6.63	2.78
Valine	16.79	7.11	3.26
Sub-total	42.14	22.97	19.57
Non-essential amino acid			
Alanine	13.77	ND	ND
Arginine	9.47	9.66	10.20
Asparagine	5.67	ND	ND
Aspartic acid	ND	11.65	7.58
Cystine	2.76	1.96	ND
Glutamic acid	5.51	24.14	18.88
Glycine	5.38	1.10	0.37
Ornithine	0.64	ND	ND
Proline	5.51	7.03	2.29
Serine	12.27	22.28	0.99
Tyrosine	6.21	3.41	3.52
Sub-total	67.19	81.23	43.83
Other free amino acid			
1-methylhistidine	ND	ND	1.20
Ammonium chloride	ND	ND	ND
Anserine	ND	ND	ND
Carnosine	ND	9.01	6.13
Citrulline	36.42	147.77	26.24
Cystathionine	ND	ND	ND
Ethanolamine	13.14	8.66	7.73
Homocysteine	142.39	27.17	4.91
Hydroxylysine	0.29	0.27	ND
Hydroxyproline	ND	ND	ND
Phosphoethanolamone	ND	ND	ND
Phosphoserine	13.32	33.92	ND
Sarcosine	3.05	2.66	ND
Taurine	ND	ND	ND
Urea	ND	ND	ND
α-aminoadipic acid	ND	ND	ND
α-aminobutyric acid	ND	ND	5.80
β-alanine	1.18	0.69	2.52
β-aminoisobutyric acid	ND	ND	ND
γ-aminobutyric acid	2.89	1.99	3.50
Sub-total	212.68	223.13	50.7
Ratio of essential to non-essential amino acids	0.63	0.28	0.45
Total	322.01	327.33	114.10

JYS = Jinyeong; YYS = Yangyangdongchulsi; and HAS = Hamanmulgam. Values are means of duplicate experiments (n = 2). ND = Not detected.

The branched-chain amino acids (leucine, isoleucine, and valine), which were also found in the seeds of three persimmon cultivars, have been reported to mediate anti-obesity effects in rodents (Lynch and Adams, 2014).

Antioxidant potential of persimmon seed

The DPPH radical, superoxide anion, and hydroxyl radical scavenging activities were considered for evaluating the antioxidant potentials of persimmon seeds. The antioxidant potentials of the persimmon seeds are indicated (Table 5) with reference to vitamin C (25 ppm).

The DPPH radical scavenging activity of HAS (71.26%) was significantly ($p < 0.05$) high, followed by JYS and YYS (Table 5). The persimmon seeds of all three cultivars showed more than 64% DPPH radical scavenging potential, indicating a potential source of antioxidants. However, the scavenging potential found in the present study was 22% lower than that found in a previous study (Ahn *et al.,* 2002). The difference in DPPH radical scavenging activity among cultivars might be owing to the difference in the amount and form of phytochemicals including tannin, the main phenolic compound of persimmon (Jang *et al.,* 2011).

The superoxide anion scavenging activities were significantly different ($p < 0.05$) among persimmon seeds of the three cultivars. It was the highest in YYS (34.38%) and the lowest in HAS (9.67%) (Table 5). Bilal *et al.* (2016) also reported a considerable amount of superoxide anion scavenging potential in persimmon seeds, which also significantly varied with cultivar. ROSs such as hydrogen peroxide, hydroxyl radicals, and singlet oxygen primarily generated from superoxide anions, induce oxidative damage in lipids, proteins, and DNA (Pietta, 2000). In the present work, the difference in the superoxide anion scavenging activity among cultivars might be attributed to the different levels of antioxidants present in persimmon seeds, which scavenge superoxide radicals by combining with superoxide radical ions to form stable radicals, thus terminating the radical chain reaction (Wang *et al.,* 2009).

The percentage inhibition of persimmon seed extracts on hydroxyl radical scavenging among cultivars was significantly different ($p < 0.05$). The order of hydroxyl radical scavenging activity of the cultivars was YYS > JYS > HAS. The hydroxyl radicals are highly reactive free radicals that are inevitably formed in biological systems and have been considered as highly damaging species in free radical pathology, capable of destroying almost every molecule found in living cells (Gülçin, 2006). The variation in hydroxyl radical scavenging potential among the cultivars might be owing to variations in their antioxidant activity. There is no specific enzyme present in the human body that can nullify the deleterious effect of hydroxyl radicals and defend against them. Therefore, antioxidants, including those from persimmon seeds, could be one potential source to scavenge these free radicals. Moreover, persimmon seeds possessed more than ten times higher ferric reducing antioxidant power than that of the pulp (Guo *et al.,* 2003).

Pharmacological properties of persimmon seed

The AChE inhibition activity of persimmon seeds was considered for investigating their pharmacological potential. All three samples showed strong anti-AChE activity (Table 5). Among the three cultivars, JYS (99.0%) possessed the highest inhibition potential, while HAS (73.9%) the lowest. Interestingly, JYS showed higher anti-AChE potential than that of the reference compound, tacrine (97.2%). The

Table 5. DPPH free radical scavenging activity, superoxide anion scavenging activity (O_2^-), hydroxyl radical scavenging activity (•OH), and acetylcholinesterase inhibition potential of seeds of three Korean persimmon cultivars.

Cultivar	% Inhibition			
	DPPH·	O_2^-	•OH	Acetylcholinesterase
JYS	67.30 ± 0.24^{b}	24.07 ± 0.07^{b}	29.29 ± 0.12^{b}	99.0 ± 0.2^{a}
YYS	64.76 ± 0.61^{c}	34.38 ± 1.21^{a}	50.66 ± 0.16^{a}	85.4 ± 1.1^{c}
HAS	71.26 ± 0.92^{a}	9.67 ± 0.23^{c}	10.00 ± 0.34^{c}	73.9 ± 0.6^{d}
Vitamin C	84.48	72.93	33.43	NA
Tacrine	NA	NA	NA	97.2 ± 0.5^{b}

JYS = Jinyeong; YYS = Yangyangdongchulsi; and HAS = Hamanmulgam. Values are expressed as mean ± SD. Different superscripts within a column indicate significant differences ($p < 0.05$). NA = not applicable.

AChE inhibition activities of persimmon seeds found in the present work were higher than those found in a previous study (Bilal *et al.,* 2016). The discrepancy in AChE inhibition activity of different cultivars was possibly owing to the genotypic variation (Ganji Moghaddam *et al.,* 2013). The level of acetylcholine in the brain can be increased by preventing the inhibitory effect of AChE. The effect of AChE inhibitors has been well-described in clinical studies to increase the endogenous level of acetylcholine and cholinergic neurotransmission in the brains of patients with Alzheimer's-type dementia. Therefore, they are regarded as highly promising therapeutic agents for Alzheimer's patients (Singhal *et al.,* 2012), glaucoma, myasthenia gravis, and for the recovery of neuromuscular block in surgery (Singhal *et al.,* 2012). It is unclear which compound contributed to the high anticholinesterase activity in the persimmon seed extract in the present work, although it has been reported that essential oils from the leaf and peel of *Citrus aurantifolia* possess anticholinesterase activity owing to the presence of active compounds, such as limonene, l-camphor, citronellol, o-cymene, and 1,8-cineole (Lee *et al.,* 2010).

Conclusion

Persimmon seeds of three cultivars were investigated for their nutritional, antioxidant, and pharmacological potentials. The results revealed that the persimmon seeds could be a potential source of different mineral elements, and do not contain any detectable trace of heavy metals such as arsenic, lead, cadmium, and mercury. The high organic acid contents of persimmon seeds increase their potential to be used as a source of natural antioxidants. Free sugars present in the persimmon seeds contributed to a well-balanced energy supply. The persimmon seeds also contained a wide variety of free amino acids. In addition, the seed extracts of persimmon were good free radical scavengers, thus indicating that the seeds could be a potential source of natural antioxidants. The present work also showed that some of the compounds present in persimmon seeds have a promising inhibitory capacity against acetylcholinesterase activity. Further studies are nevertheless required to evaluate the therapeutic potential of persimmon seeds against Alzheimer's disease. Persimmon seeds have antioxidant, nutritional, and pharmacological values, and could be utilised in the food and pharmaceutical industries.

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