Kor. J. Hort. Sci. Technol. 31(2):231-238, 2013 DOI http://dx.doi.org/10.7235/hort.2013.12177

Characterization of Lipophilic Nutraceutical Compounds in Seeds and Leaves of *Perilla frutescens*

Seungduk Um[1,2,3†], Shiva Ram Bhandari[4,5†], Nam-Hoon Kim[1,2,3], Tae-Jin Yang[1,2,3], Ju Kyoung Lee[6,7], and Young-Sang Lee[4*]

[1]*Department of Plant Science, Seoul National University, Seoul 151-921, Korea*
[2]*Plant Genomics and Breeding Institute, Seoul National University, Seoul 151-921, Korea*
[3]*Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Korea*
[4]*Department of Medical Biotechnology, Soonchunhyang University, Asan 336-745, Korea*
[5]*Department of Horticultural Crop Research, National Institute of Horticultural & Herbal Science, Rural Development Administration, Suwon 440-706, Korea*
[6]*Department of Applied Plant Sciences, Kangwon National University, Chuncheon 200-701, Korea*
[7]*College of Agriculture and Life Sciences, Kangwon National University, Chuncheon 200-701, Korea*

Abstract. *Perilla frutescens*, which comprises var. *frutescens* and var. *crispa*, has been cultivated traditionally in Asian countries as an edible oil, leaf vegetable, and medicinal crop. To evaluate the lipophilic phytonutrient properties of *P. frutescens*, we selected 54 *Perilla* accessions [19 landraces of var. *frutescens* (FL), 22 weedy type var. *frutescens* (FW), 9 weedy type var. *crispa* (CW), 2 cultivars of var. *frutescens* widely cultivated for seed oil (FCS), and 2 cultivars of var. *frutescens* cultivated as a leaf vegetable (FCL)] and analyzed their seeds and leaves for vitamin E, squalene, and phytosterols. Among the four vitamin E isomers analyzed, γ-tocopherol was the major form of vitamin E in seeds, whereas α-tocopherol was the major form in leaves of all types of *P. frutescens*. The highest total vitamin E content in seeds was present in FL (170.0 $mg \cdot kg^{-1}$), whereas that in leaves was highest in FCL (358.1 $mg \cdot kg^{-1}$). The highest levels of squalene in seeds and leaves were in FL (65.5 $mg \cdot kg^{-1}$) and CW (719.3 $mg \cdot kg^{-1}$), respectively. Among the three phytosterols, β -sitosterol occurred in the highest amount in both leaves and seeds of all of the crop types. Phytonutrient contents were comparatively higher in leaves than in seeds of all crop types. All of these results suggest that the consumption of leaves and seeds of *Perilla* crops could be beneficial to human health, as *Perilla* possesses considerable amounts of various lipophilic compounds.

Additional key words: *Perilla* var. *crispa*, phytosterol, squalene, vitamin E

Introduction

Perilla frutescens (L.) Britt. (Labiatae) is a self-fertilizing annual plant that is widely cultivated and distributed in the Himalayan hills, Southeast Asia, and East Asia (Makino, 1961; Nitta, 2001). Recently, it has been also grown in various other countries, including the United States, European nations, and Russia (Nitta and Ohnishi, 1999; Nitta et al., 2003). The species includes two cultivated varieties of *Perilla* that are characterized on the basis of morphology and use. *P. frutescens* var. *frutescens* is an oil crop, which is known as *ren* in Chinese, *deulkkae* in Korean, and *egoma* in Japanese, whereas *P. frutescens* var. *crispa* is a Chinese medicinal or vegetable crop called *zisu* in Chinese, *cha-jo-ki* in Korean, and *shiso* in Japanese (Lee et al., 2002). In addition to those two cultivated types of *Perilla*, weedy plants of both var. *frutescens* and var. *crispa* (Lee and Ohnishi, 2001; Nitta and Ohnishi, 1999) are commonly found in habitats such as roadsides, wastelands, abandoned fields, and marginal lands around farm fields in East Asia. The two cultivated types of

***Corresponding author:** mariolee@sch.ac.kr
[†]These authors contributed equally to this research work.
※ Received 13 September 2012; Revised 27 November 2012; Accepted 29 November 2012. This work was supported in part by the Soonchunhyang University Research Fund (20110694: Reveleris iES Flash Chromatography).

Perilla are distinguished by several morphological features. *P. frutescens* var. *frutescens* is tall and has large seeds (> 2 mm), which are either soft or hard, green leaves and stems, unwrinkled leaves, and a fragrance specific to var. *frutescens*. In contrast, *P. frutescens* var. *crispa* is short and has small seeds (< 2 mm), which are hard, either red or green leaves and stems, either wrinkly or non-wrinkly leaves, and a fragrance specific to var. *crispa* (Lee and Ohnishi, 2001). The plant is occasionally found in a relict form in Korea (Lee and Ohnishi, 2001). *P. frutescens* var. *frutescens* is widely cultivated and used in Korea (Lee et al., 2002; Nitta, 2001; Nitta et al., 2003) and believed to have originated in southern China, where a wild type of this plant is widely distributed (Lee and Ohnishi, 2003; Makino, 1960; Nitta et al., 2003). In Korea, var. *frutescens* has been one of the most important oil crops since ancient times. Var. *frutescens* has been a vegetable crop, and its seed oil has been used as both edible and industrial oil, including for lamp oil, waterproofing umbrella paper and other rain gear, and as a lacquer for wooden furniture, wooden vessels, and other household items (Choi, 1984). The seeds of var. *frutescens* have also been used as a folk medicine for inveterate gastritis and cough and as a restorative (Choi, 1984). Recently, considerable attention has been given to the anti-inflammatory, anti-allergic, and anti-tumor promoting substances contained in beefsteak plants (Makino et al., 2003; Ueda et al., 2002). With the increase of meat consumption and development of various methods for cooking the fresh leaves, var. *frutescens* is used not only as a seed oil crop but also as a leafy vegetable crop and has become one of the most important crops in Korea.

Vitamin E, phytosterols, and squalene are the chief phytonutrients found in the unsaponifiable lipid fraction of crop seeds. Vitamin E, which consists of the four isomers α-, β-, γ-, and δ- tocopherol, is a well-known natural antioxidant. By inhibiting membrane lipid peroxidation and scavenging reactive oxygen species (Kruk et al., 2005; Munne-Bosch and Alegre, 2002; Trebst et al., 2002), vitamin E is believed to reduce the risk of cardiovascular diseases and certain types of cancers (Burton, 1994; Burton and Traber, 1990). Phytosterols, primarily β-sitosterol, campesterol, and stigmasterol, are integral components of plant cell membranes and are found abundantly in vegetable oils, nuts, seeds, and grains (Weihrauch and Gardner, 1978). Phytosterols are mainly known for lowering serum cholesterol (Marangoni and Poli, 2010) and preventing the development of colon cancer (Awad and Fink, 2000) and for their atherosclerotic (Moghadasian et al., 1999), anti-inflammatory (Bouic, 2001), and anti-oxidative effects (van Rensburg et al., 2000). Squalene, a hydrocarbon, is a key intermediate in cholesterol biosynthesis (Moreda et al., 2001) and is abundant in shark liver oil (*Squalus* spp.) and olive oil. It possesses antitumor, anti-proliferative, and serum-cholesterol-lowering effects (Khor and Chieng, 1997), along with chemopreventive activity against colon carcinogenesis (Rao et al., 1998).

In this study, we collected various germplasms of *Perilla* crops and weedy types from different places in Korea and evaluated vitamin E isomers, squalene, and phytosterols in seeds and leaves to develop a highly nutritive variety.

Materials and Methods

Plant Materials

The materials used in this study consisted of 54 *Perilla* accessions, including 19 landrace accessions of var. *frutescens* (FL), 22 accessions of weedy type var. *frutescens* (FW), 9 accessions of weedy type var. *crispa* (CW), and 4 cultivars of var. *frutescens*, of which 2 were leafy vegetable crops (FCL) and 2 were seed oil crops (FCS). The 54 accessions of cultivated and weedy *Perilla* types (Table 1) were collected in Korea during October-November 2007 and 2008. We visited more than 30 villages and interviewed numerous farmers about the cultivation and utilization of *Perilla* crops. We also searched for natural distributions of weedy types of *Perilla* in farm fields, roadsides, wastelands, abandoned fields, and areas around farmhouses. The collected *Perilla* samples were classified into cultivated or weedy types based on morphological characters and cultivation conditions (cultivated or not). Four widely cultivated cultivars of var. *frutescens* developed by the National Institute of Crop Science, Rural Development Administration of Korea, were used as references. To propagate the seeds of the 54 accessions of cultivated and weedy *Perilla* types, five individuals of each accession were grown in a field at the Faculty of Agriculture, Kangwon National University, Chuncheon, Gangwon Province. For analysis in this study, 10 g of seeds harvested in 2007 from each accession was used. To analyze the leaf constituents and correlate these with their quantities in seeds, the same

Table 1. Seeds of *Perilla frutescens* used in this study.

Crop types	Accession number
var. *frutescens* weedy (FW)	**P07-2**, P07-5, P07-10, **P07-13**, **P07-15**, **P07-18**, P07-19, **P07-20**, P07-27, **P07-48**, P07-57, P07-58, P07-74, P07-83, **P07-87**, P07-112, P07-114, P07-115, **P07-128**, **P07-131**, P07-133, **P07-160**
var. *frutescens* landrace (FL)	**P07-7**, **P07-16**, **PO7-37**, P07-39, **P07-40**, **P07-45**, **P07-49**, **P07-59**, **P07-81**, **P07-122**, **P07-126**, **P07-129**, **P07-134**, **P07-135**, **P07-137**, **P07-142**, **P07-147**, **P07-153**, **P07-162**
var. *crispa* weedy (CW)	P07-17, P07-22, P07-24, P07-138, P07-143, P07-144, **P07-145**, **P07-155**, **P07-156**
var. *frutescens* cultivated for seeds (FCS)	2 cultivars for seed purpose (cv. *Perilla* Youngho and cv. *Perilla* Anyu)
var. *frutescens* cultivated for leaves (FCL)	2 cultivars for leaf purpose (cv. *Perilla* Namcheon and cv. *Perilla* Dongle-1-ho)

Note: Boldface indicates that leaves were also used in the analysis.

accessions were planted by direct seeding in June 2008 on the agricultural research farm of Seoul National University, Suwon, Korea. In September, 40 g of healthy leaves was harvested from 35 well-grown accessions and lyophilized using a freeze-dryer (IlShin BioBase, Korea). The seeds and leaves were then ground into fine powder and stored at -20°C for further analysis.

Sample Preparation for Analysis of Tocopherols, Phytosterols, and Squalene

Samples for the analysis of tocopherols, phytosterols, and squalene were prepared according to Bhandari et al. (2012) with some modifications. Dry powdered *Perilla* seeds and leaves (1.0 g) were placed in a 50 mL Falcon tube and extracted with 15 mL ethanol using 100 mg ascorbic acid as an antioxidant. The mixture was shaken in a hot-water bath at 80°C for 10 min. Then, for saponification, 300 μL 44% potassium hydroxide was added, and the mixture was shaken for 18 min under the same conditions. After saponification, each tube was cooled in ice, and 10 mL of n-hexane and 10 mL of distilled water were added and mixed, and the mixture was centrifuged at 1900 × *g* for 10 min. The upper hexane layer was then collected, and 10 mL *n*-hexane was added to the remaining solution. This process was repeated three times, with the collected hexane solutions pooled and then washed three times with 10 mL distilled water. The solutions were passed through Na_2SO_4 anhydrous to remove water, concentrated in a rotary evaporator, and redissolved in 1 mL isooctane.

Analysis of Tocopherols, Squalene, and Phytosterols

Four vitamin E isomers (α-, β-, γ-, and δ- tocopherols), squalene, and three phytosterols (campesterol, stigmasterol, and β-sitosterol) were quantified using a gas chromatograph (Varian 3800; Varian Inc., Palo Alto, CA, USA) according to Bhandari et al. (2012) The analysis was performed using a CP-SIL 8CB capillary column (25.0 m × 250 μm × 0.40 μm nominal) at a constant flow rate (1.0 $mL \cdot min^{-1}$) of helium as a carrier gas. The injection volume was 1 μL, and the split ratio was 1:20. The temperature for the injector and flame ionization detector was set at 290°C. The column oven temperature was initially set at 220°C for 2 min, then raised to 290°C at a rate of $5°C \cdot min^{-1}$ and held for 14 min, after which the temperature was raised to 310°C. All of the vitamin E, squalene, and phytosterols peaks were identified by comparison to the retention time of authentic standard peaks.

Standard Solution Preparation

External standard solutions of the four vitamin E isomers were prepared by dissolving the authentic standards separately in isooctane to prepare 1,000 ppm stock solutions. Later, they were mixed and diluted to proper concentrations. Similarly, standard stock solutions of squalene and the phytosterols were separately dissolved in isooctane and chloroform, respectively, and further diluted to the desired concentrations.

Chemicals and Reagents

Vitamin E standards (α-, β-, γ-, and δ-tocopherol) were purchased from Merck (Darmstadt, Germany). Squalene, stigmasterol, β-sitosterol, and campesterol were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ascorbic acid, chloroform, and anhydrous sodium sulfate were acquired from Samchun (Seoul, Korea), potassium hydroxide was purchased from Daejung (Seoul, Korea), and ethanol was obtained from Duksan Chemicals (Seoul, Korea). Hexane (HPLC grade) and isooctane (2,2,4-trimethyl pentane; HPLC grade) were purchased from J.T. Baker (Phillipsburg, NJ, USA).

Statistical Analysis

For quantitative studies, at least three independent sample extraction replications were conducted. Descriptive statistical analyses were performed using SPSS (ver. 18; SPSS, Inc., Chicago, IL, USA) at a significance level of $P = 0.05$.

Results and Discussions

Vitamin E Content

Vitamin E analysis of all types of *P. frutescens* evaluated in this study showed that only tocopherols were present in both seeds and leaves (Table 2). Seeds of all types of *P. frutescens* exhibited the highest γ-tocopherol content among the four tocopherol isomers (α-, β-, γ-, and δ-tocopherol). Similar results have previously been observed in *Perilla* seeds (Park et al., 2004; Shin and Kim, 1994), however, in these cases, only one variety was used, and the name was not clearly identified. FL showed a higher level of seed vitamin E (170.1 mg·kg^{-1}) than FW, CW, or the two var. *frutescens* cultivars developed for seed (FCS) and leaf (FCL) purposes by the National Institute of Crop Science of the Rural Development Administration of Korea. Among accessions of the same type, highest total vitamin E content in seed was observed in accessions PO7-128 (204.9 mg·kg^{-1}), PO7-59 (203.3 mg·kg^{-1}), PO7-24 (161.0 mg·kg^{-1}) in FW, FL, and CW types, respectively. The average seed vitamin E content in *Perilla* crops (152.1 mg·kg^{-1}) was higher than that in various other seed oil crops, such as linseed (83.0 mg·kg^{-1}), mustard (69.0 mg·kg^{-1}), and sesame (100.0 mg·kg^{-1}) (Ryan et al., 2007). Similarly, Bhandari et al. (2011) reported 132.5 mg·kg^{-1} total vitamin E content in seeds of 12 var. *crispa* landraces collected in Korea and Japan. In contrast to the varying γ-tocopherol

Table 2. Descriptive statistics of vitamin E isomer contents (mg·kg^{-1}) in seeds and leaves of *Perilla frutescens*.

Crop Type	Descrip-tives	Seeds					Leaves			
		α-T[z]	β-T	γ-T	δ-T	Total -T	α-T	β-T	γ-T	Total-T
var. *frutescens* weedy type (FW)	Minimum	2.7	1.3	112.1	2.4	126.5	62.3	0.9	0.6	63.8
	Maximum	12.1	10.7	187.5	6.1	204.9	364.2	2.4	4.1	368.1
	Mean	6.8	5.7	135.4	4.6	150.1	244.6	1.6	1.4	247.7
	SD[y]	3.1	2.5	18.7	1.0	18.8	98.9	0.6	1.0	100.0
	CV (%)[x]	45.6	43.9	13.8	21.7	12.5	40.4	37.5	71.4	40.4
var. *frutescens* landrace (FL)	Minimum	8.5	1.4	124.5	2.5	140.5	172.0	0.9	0.8	173.6
	Maximum	13.3	8.9	189.9	6.2	203.3	614.0	3.2	4.9	620.0
	Mean	10.3	5.0	151.9	4.5	170.0	308.0	2.0	1.8	311.7
	SD	1.4	3.1	21.7	1.1	21.0	113.3	0.5	0.9	114.2
	CV (%)	13.6	62.0	14.3	24.4	12.4	36.8	25.0	50.0	36.6
var. *crispa* weedy type (CW)	Minimum	4.4	1.8	105.2	3.4	117.6	243.4	1.5	0.6	245.5
	Maximum	6.9	10.1	137.9	6.7	161.0	318.6	2.1	1.8	322.5
	Mean	6.0	4.1	120.9	4.7	135.7	274.9	1.7	1.4	278.1
	SD	0.8	3.1	11.8	1.2	14.7	39.0	0.3	0.7	39.8
	CV (%)	13.3	75.6	9.8	25.5	10.8	14.2	17.6	50.0	14.3
FCS	Mean	5.7	1.2	134.6	3.7	145.2	291.2	2.0	2.2	295.3
	SD	0.2	0.2	5.5	0.3	6.2	92.1	0.4	0.3	92.8
FCL	Mean	11.1	1.3	144.8	2.3	159.4	354.0	2.1	2.1	358.1
	SD	0.1	0.1	12.1	1.7	13.9	112.2	0.3	0.8	111.7
Mean		8.0	3.5	137.5	4.0	152.1	294.5	1.9	1.8	298.2

[z]T: Tocopherol.
[y]SD: Standard deviation.
[x]CV: Coefficient of variation.

levels in seeds, leaves of all types of *P. frutescens* had the highest content of α-tocopherol among three tocopherols (α-, β- and γ-tocopherol) quantified. Compared to seeds, leaves of all crop types showed higher total vitamin E contents. The average leaf vitamin E content was 298.2 mg·kg^{-1}, which was higher than that in various vegetable crops, including broccoli (17.7 ± 3.4 mg·kg^{-1}) and asparagus (12.4 mg·kg^{-1}) (Chun et al., 2006). We observed the highest leaf vitamin E content in FCL (358.1 mg·kg^{-1}) among the accessions of cultivated and weedy types of *Perilla* analyzed in this study, although this cultivar was not intentionally bred for high vitamin E content. Among accessions of the same type, accessions PO7-87 (368.1 mg·kg^{-1}), PO7-59 (620 mg·kg^{-1}) and PO7-156 (322.5 mg·kg^{-1}) exhibited highest leaf vitamin E in FW, FL, and CW types, respectively.

Squalene Content

Squalene, a precursor of phytosterol biosynthesis, has an important role in lowering cholesterol, as well as chemopreventive effects, but is rarely studied in *Perilla* crops. In our study, we found a considerable seed squalene ranging from 16.8 to 85.1 mg·kg^{-1} compared to that reported in other crops, such as mustard (5.0 ± 0.5 mg·kg^{-1}), sesame (6.0 ± 0.4 mg·kg^{-1}), and linseed (10.0 ± 0.4 mg·kg^{-1}) (Ryan et al., 2007) (Table 3). Highest seed squalene content in various *Perilla* types; FW, FL, and CW was 77.7 mg·kg^{-1} (Acc. No.: PO7-20), 85.1 mg·kg^{-1} (Acc. No.: PO7-7) and 161.0 mg·kg^{-1} (Acc. No.: PO7-24), respectively. FL (65.5 mg·kg^{-1}) had the highest average squalene content in seeds, followed by FW (46.7 mg·kg^{-1}), FCL (44.5 mg·kg^{-1}), FCS (29.6 mg·kg^{-1}), and CW (28.4 mg·kg^{-1}). *Perilla* leaves exhibited higher squalene content than the seeds, with CW exhibiting the highest average squalene content (719.3 mg·kg^{-1}) among all *P. frutescens* types. The accessions with highest leaf squalene content among FW, FL, and CW types were PO7-48 (769.7 mg·kg^{-1}), PO7-135 (947.4 mg·kg^{-1}), and PO7-156 (980.2 mg·kg^{-1}),

Table 3. Descriptive statistics of squalene and phytosterols (campesterol, stigmasterol, and β-sitosterol) contents (mg·kg^{-1}) in seeds and leaves of *Perilla frutescens*.

Crop Type	Descriptives	Seeds					Leaves				
		Squalene	Campe-sterol	Stigma-sterol	β-sitosterol	Total phytosterol	Squalene	Campe-sterol	Stigma-sterol	β-sitosterol	Total phytosterol
var. *frutescens* weedy type (FW)	Minimum	16.8	18.2	25.4	393.4	442.0	141.5	42.6	73.3	740.5	872.0
	Maximum	77.7	48.8	50.4	593.6	652.0	769.7	68.0	135.9	950.6	1087.7
	Mean	46.7	33.7	37.7	477.2	548.6	497.9	59.6	100.2	847.4	1007.2
	SD^z	18.3	12.1	7.0	60.0	60.1	214.8	8.3	23.3	65.2	64.3
	CV^x(%)	39.2	35.9	18.6	12.6	11.0	43.1	13.9	23.3	7.7	6.4
var. *frutescens* landrace (FL)	Minimum	50.8	21.4	22.3	461.0	507.4	227.7	45.7	73.8	598.3	721.6
	Maximum	85.1	48.5	47.7	607.8	671.0	947.4	81.4	113.8	1068.8	1243.2
	Mean	65.5	33.3	32.1	523.5	588.9	591.2	65.0	94.5	861.1	1020.7
	SD	10.1	11.1	8.0	42.2	46.7	239.4	9.1	12.1	108.3	119.8
	CV (%)	15.4	33.3	24.9	8.1	7.9	40.5	14.0	12.8	12.6	11.7
var. *crispa* weedy type (CW)	Minimum	25.3	16.6	29.7	355.0	401.2	496.1	64.1	90.7	902.5	1057.3
	Maximum	35.7	48.5	54.3	466.7	557.4	980.2	74.9	159.9	970.3	1205.1
	Mean	28.4	28.0	38.8	412.3	478.9	719.3	70.8	124.4	931.7	1126.9
	SD	3.2	13.6	9.3	32.9	45.8	244.2	5.9	34.6	34.9	74.3
	CV (%)	11.3	48.6	24.0	8.0	9.6	33.9	8.3	27.8	3.7	6.6
FCS	Mean	29.6	16.6	23.5	440.2	480.2	100.8	34.4	54.1	614.7	703.2
	SD	4.9	1.7	6.7	90.7	95.7	32.9	7.2	1.3	4.7	13.2
FCL	Mean	44.5	19.5	28.5	459.4	507.3	176.9	37.2	75.7	584.6	697.6
	SD	5.4	1.8	5.2	19.9	12.9	39.0	2.1	0.8	16.3	14.9
Mean		42.9	26.2	32.1	462.5	520.8	417.2	53.4	89.8	767.9	911.1

^{z}SD: Standard deviation.
^{x}CV: Coefficient of variation.

respectively. The presence of a high quantity of squalene enriches the medicinal value of *Perilla* crops, as it possesses antitumor, anti-proliferative, and serum-cholesterol-lowering effects (Khor and Chieng, 1997). Thus, both *Perilla* seeds and leaves could be good sources of squalene, thereby enriching its pharmacological value.

Phytosterol Content

Three major phytosterols, campesterol, stigmasterol, and β-sitosterol, were analyzed in both the seeds and leaves of the different *P. frutescens* types. In both the leaves and seeds of all types, β-sitosterol was present in highest quantity, followed by campesterol and stigmasterol (Table 3), which was similar to previous studies on different plants (Normen et al., 1999; Ryan et al., 2007; Weihrauch and Gardner, 1978). The highest levels of campesterol, stigmasterol, and β-sitosterol in seeds were present in types FW (33.7 mg·kg^{-1}), CW (38.8 mg·kg^{-1}), and FL (523.5 mg·kg^{-1}), respectively. Both cultivated and weedy types of var. *frutescens* accessions exhibited higher seed total phytosterol (548.6 mg·kg^{-1} in FL, and 588.9 mg·kg^{-1} in FW) compared to CW, FCS, and FCL. Similarly, highest seed total phytosterol was present in PO7-128 (652.0 mg·kg^{-1}), PO7-39 (671.0 mg·kg^{-1}), PO7-24 (557.4 mg·kg^{-1}) accessions of FW, FL, and CW types, respectively.

In leaves, all accessions of cultivated and weedy types of *Perilla* crops showed higher total phytosterol, as did each of the corresponding phytosterols, than levels in seeds. CW exhibited the highest campesterol (70.9 mg·kg^{-1}), stigmasterol (124.4 mg·kg^{-1}), and β-sitosterol (931.6 mg·kg^{-1}), as well as total phytosterol content (1126.9 mg·kg^{-1}) than other types. *Perilla* accessions: PO7-15 (1087.7 mg·kg^{-1}), PO7-49 (1243.2 mg·kg^{-1}) and PO7-155 (1205.1 mg·kg^{-1}) exhibited highest amount of total phytosterols among the accessions of FW, FL, and CW types, respectively. The presence of considerable amounts of phytosterols in both the leaves and seeds of two cultivated types of *Perilla* increases its medicinal importance, as these compounds have diverse physiological as well as pharmacological activities, such as cholesterol-lowering (Jones et al., 1997) and anti-tumor effects (Raicht et al., 1980).

Correlations among Phytonutrients

To understand the accumulation pattern of phytonutrients as well as to examine the possibility of breeding a phytonutrient-enriched variety of *P. frutescens*, the correlationships among lipophilic phytonutrients in seeds or in leaves were examined for each type of *P. frutescens*. In seeds, total vitamin E was strongly and positively correlated with squalene in weedy type of FW (r = 0.723**) and CW (r = 0.871**), but was not correlated with that in the case of FL (Table 4). Likewise, vitamin E content also showed a positive correlationship with β-sitosterol in FW and FL. Among seed phytonutrients analyzed, β-sitosterol exhibited highest

Table 4. Correlation coefficients among lipophilic phytonutrient contents in *Perilla frutescens* seeds.

Crop Type	Phytonutrients	Squalene	Campesterol	Stigmasterol	β-sitosterol	Total phytosterols
var. *frutescens* weedy type (FW)	Total vitamin E	0.723**	-0.141	-0.289	0.848**	0.784**
	Squalene		0.178	-0.225	0.656**	0.665**
	Campesterol			0.570**	-0.006	0.261
	Stigmasterol				-0.318	-0.087
	β-sitosterol					0.960**
var. *frutescens* landrace (FL)	Total vitamin E	0.439	-0.035	0.003	0.848**	0.758**
	Squalene		0.259	0.185	0.473*	0.521*
	Campesterol			0.855**	-0.007	0.378
	Stigmasterol				0.096	0.461*
	β-sitosterol					0.919**
var. *crispa* weedy type (CW)	Total vitamin E	0.871**	0.511	0.583	0.610	0.708*
	Squalene		0.590	0.505	0.594	0.704*
	Campesterol			0.883**	0.406	0.767*
	Stigmasterol				0.260	0.651
	β-sitosterol					0.891**

*,**Correlation is significant at the P = 0.05 and P = 0.01 levels, respectively.

Table 5. Correlation coefficients among lipophilic phytonutrient contents in *Perilla frutescens* leaves.

Crop type	Phytonutrients	Squalene	Campesterol	Stigmasterol	β-sitosterol	Total phytosterols
var. *frutescens* weedy type (FW)	Total vitamin E	0.748*	0.503	0.358	-0.241	-0.050
	Squalene		0.731*	0.441	-0.291	-0.042
	Campesterol			0.576	-0.330	0.003
	Stigmasterol				-0.199	0.235
	β-sitosterol					0.901**
var. *frutescens* landrace (FL)	Total vitamin E	0.300	0.472*	0.484*	0.312	0.366
	Squalene		0.299	0.069	0.448	0.435
	Campesterol			0.331	0.879**	0.903**
	Stigmasterol				0.234	0.337
	β-sitosterol					0.994**

*,**Correlation is significant at the P = 0.05 and P = 0.01 levels, respectively.

positive correlationship with total phytosterol content in all types of *Perilla* mainly due to its high compositional ratio among phytosterols. However, squalene, a precursor of phytosterols, showed positive relationship only with β-sitosterol in FW and FL, but not in CW. Compared to seeds, phytonutrients in leaves exhibited relatively low correlations (Table 5) with β-sitosterol, which although showed positive correlationship with total phytosterol content in both FW (r = 0.901*) and FL (r = 0.994*).

In summary, this study demonstrated that both seeds and leaves of *Perilla* may be good sources of vitamin E, squalene, and phytosterols. Among the four vitamin E isomers present in seeds, γ-tocopherol was found in the highest quantity in all of the *Perilla* types tested. Likewise, leaves exhibited higher total vitamin E content than seeds in all cases. Similarly, β-sitosterol was more abundant than other forms of phytosterols in both seeds and leaves, and as in vitamin E, total phytosterol content was higher in leaves than in seeds. Comparatively, seeds of *Perilla* crops exhibited higher correlations among phytonutrients than leaves. This is the first study to provide the lipophilic nutritional value of various types of *P. frutescens* and to suggest a possible enhancement in phytonutrient values by using both leaves and seeds.

Literature Cited

Awad, A.B. and C.S. Fink. 2000. Phytosterols as anticancer dietary components: evidence and mechanism of action. J. Nutr. 130:2127-2130.

Bhandari, S.R., J.K. Lee, and Y.S. Lee. 2011. Phytonutrient profile of purple perilla (*Perilla frutescens* var. *crispa*) seeds. Korean J. Crop Sci. 56:199-204.

Bhandari, S.R., S. Basnet, K.H. Chung, K.H. Ryu, and Y.S. Lee. 2012. Comparisons of nutritional and phytochemical property of genetically modified CMV-resistant red pepper and its parental cultivar. Hort. Environ. Biotechnol. 53:151-157.

Bouic, P.J. 2001. The role of phytosterols and phytosterolins in immune modulation: A review of the past 10 years. Curr. Opin. Clin. Nutr. Metab. Care 4:471-475.

Burton, G.W. and M.G. Traber. 1990. Vitamin E: Antioxidant activity, biokinetics, and bioavailability. Annu. Rev. Nutr. 10:357-382.

Burton, G.W. 1994. Vitamin E: Molecular and biological function. Proc. Nutr. Soc. 53:251-262.

Choi, Y.J. 1984. Deulkkae (*Perilla frutescens* var. *frutescens*): Story of folk custom in Korea. New Hort. 26:52-53.

Chun, J., J. Lee, L. Ye, J. Exler, and R.R. Eitenmiller. 2006. Tocopherol and tocotrienol contents of raw and processed fruits and vegetables in the United States diet. J. Food Comp. Anal. 19:196-204.

Khor, H.T. and D.Y. Chieng. 1997. Effect of squalene, tocotrienols and α-tocopherol supplementations in the diet on serum and liver lipids in hamsters. Nutr. Res. 17:475-483.

Kruk, J., H. Hollander-Czytko, W. Oettmeier, and A. Trebst. 2005. Tocopherol as singlet oxygen scavenger in photosystem II. J. Plant Physiol. 162:749-757.

Jones, P.J., D.E. Macdougall, F. Ntanios, and C.A. Vanstone. 1997. Dietary phytosterols as cholesterol-lowering agents in humans. Can. J. Physiol. Pharmacol. 75:217-227.

Lee, J.K. and O. Ohnishi. 2001. Geographical differentiation of morphological characters among *Perilla* crops and their weedy types in East Asia. Breed Sci. 51:247-255.

Lee, J.K. and O. Ohnishi. 2003. Genetic relationships among cultivated types of *Perilla frutescens* and their weedy types in East Asia revealed by AFLP markers. Genet. Resour. Crop Evol. 50:65-74.

Lee, J.K., M. Nitta, N.S. Kim, C.H., Park, K.M. Yoon, Y.B. Shin, and O. Ohnishi. 2002. Genetic diversity of *Perilla* and related weedy types in Korea determined by AFLP analyses. Crop Sci. 42:2161-2166.

Makino, T. 1961. Makino's new illustrated flora of Japan. Hokuryukan Pub., Tokyo, Japan.

Makino, T., Y. Furuta, H. Wakushima, H. Fujii, K. Saito, and Y. Kano. 2003. Anti-allergic effect of *Perilla frutescens* and its active constituents. Phytother. Res. 17:240-243.

Marangoni, F. and A. Poli. 2010. Phytosterol and cardiovascular health. Pharmacol. Res. 61:193-199.

Moghadasian, M.H., B.M. McManus, D.V. Godin, R. Rodrigues, and J.J. Frohlich. 1999. Proatherogenic and antiantherogenic effects of probucol and phytosterols in apolipoprotein E-deficient mice: Possible mechanisms of action. Circulation 99:1733-1739.

Moreda, W., M.C. Perez-Camino, and A. Cert. 2001. Gas and liquid chromatography of hydrocarbons in edible vegetable oils. J. Chromatogr. A 936:159-171.

Munne-Bosch, S. and L. Alegre. 2002. The function of tocopherols and tocotrienols in plants. Crit. Rev. Plant Sci. 21:31-57.

Nitta, M. and O. Ohnishi. 1999. Genetic relationships among two *Perilla* crops, *shiso* and *egoma*, and the weedy type revealed by RAPD markers. Genes. Genet. Syst. 74:43-48.

Nitta, M. 2001. Origin of *Perilla* crops and their weedy type. Ph.D. dissertation, Kyoto University, Kyoto, Japan.

Nitta, M., J.K. Lee, and O. Ohnishi. 2003. Asian *Perilla* crops and their weedy forms: Their cultivation, utilization and genetic relationships. Econ. Bot. 57:245-253.

Normen, L., M. Johnsson, H. Adersson, Y. Van Gameren, and P. Dutta. 1999. Plant sterols in vegetables and fruits commonly consumed in Sweden. Eur. J. Nutr. 38:84-89.

Park, K.Y., C.S. Kang, Y.S. Lee, Y.H. Lee, and Y.S. Lee. 2004. Tocotrienol and tocopherol content in various plant seeds. Korean J. Crop Sci. 49:207-210.

Raicht, R.F., B.I. Cohen, E.P. Fazzini, A.N. Sarwal, and M. Takahashi. 1980. Protective effect of phytosterols against chemically induced colon tumors in rats. Cancer Res. 40:403-405.

Rao, C.V., H.L. Newmark, and B.S. Reddy. 1998. Chemopreventive effect of squalene on colon cancer. Carcinogenesis 19:287-290.

Ryan, E., K. Galvin, T.P. O'Connor, and A.R. Maguire. 2007. Phytosterol, squalene, tocopherol content and fatty acid profile of selected seeds, grains, and legumes. Plant Foods Hum. Nutr. 62:85-91.

Shin, H.S. and S.W. Kim. 1994. Lipid composition of *Perilla* seed. J. Am. Oil Chem. Soc. 71:619-622.

Trebst, A., B. Depka, and H. Hollander-Czytko. 2002. A specific role for tocopherol and of chemical singlet oxygen quenchers in the maintenance of photosystem II structure and function in *Chlamydomonas reinhardtii*. FEBS Lett. 516:156-160.

Ueda, H., C. Yamazaki, and M. Yamazaki. 2002. Luteolin as an anti-inflammatory and anti-allergic constituent of *Perilla frutescens*. Biol. Pharm. Bull. 25:1197-1202.

van Rensburg, S.J., W.M. Daniels, J.M. van Zyl, and J.J. Taljaard. 2000. A comparative study of the effects of cholesterol, β-sitosterol, glucoside, dehydroepiandrosterone sulphate and melatonin on in vitro lipid peroxidation. Metab. Brain Dis. 15:257-265.

Weihrauch, J.L. and J.M. Gardner. 1978. Sterol content of foods of plant origin. J. Am. Diet. Assoc. 73:39-44.