Lipid Composition of Perilla Seed

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The composition of lipids and oil characteristics from perilla [Perilla frutescens (L.) Britt.] seed cultivars are reported. Total lipid contents of the five perilla seed cultivars ranged from 38.6 to 47.8% on a dry weight basis. The lipids consisted of 91.2-93.9% neutral lipids, 3.9-5.8% glycolipids and 2.0-3.0% phospholipids. Neutral lipids consisted mostly of triacylglycerols (88.1-91.0%) and small amounts of sterol esters, hydrocarbons, free fatty acids, free sterols and partial glycerides. Among the glycolipids, esterified sterylglycoside (48.9-53.2%) and sterylglycoside (22.1-25.4%) were the most abundant, while monogalactosyldiacylglycerol and digalactosyldiacylglycerol were present as minor components. Of the phospholipids, phosphatidylethanolamine (50.4-57.1%) and phosphatidylcholines (17.6–20.6%) were the major components, and phosphatidic acid, lysophosphatidylcholine, phosphatidylserine and phosphatidylinositol were present in small quantities. The major fatty acids of the perilla oil were linolenic (61.1– 64.0%), linoleic (14.3-17.0%) and oleic acids (13.2-14.9%). Some of the physicochemical characteristics and the tocopherol composition of perilla oil were determined.

KEY WORDS: Perilla frutescens, perilla oil, perilla seed.

Perilla [*Perilla frutescens* (L.) Britt.] has been mainly cultivated for edible purposes in Korea since ancient times. The seeds are consumed as flavoring and nutritional sources in combination with cereals or vegetables after roasting. Intact leaves are also used as condiments or flavoring agents in various Korean foods. Perilla oil is widely used as a salad oil or cooking medium. Annual production of perilla seed is approximately 40,000 metric tons, and the expelled oil production is the third largest among edible oils produced in Korea.

Determination of the components of lipid classes and fatty acid composition of perilla seed may be useful in understanding membrane composition, oxidative stability and nutritional roles of important fatty acids. However, systematic studies of the contents and composition of individual components are still scanty, although the examination of chemical and nutritional aspects on perilla seed has been reported (1). The objective of this study was to determine the lipid profile, in terms of lipid classes, fatty acid composition and physicochemical characteristics of five different perilla varieties grown in Korea.

EXPERIMENTAL PROCEDURES

Five varieties of mature perilla seed were obtained from the Crops Testing Center of the Agricultural Development Office of South Korea (Suwon, South Korea). The samples of seed were harvested there at the Crops Testing Center in 1991 and stored at 4°C until needed. Pure neutral lipids (NL), glycolipids (GL), phospholipids (PL) and fatty acid methyl esters (FAME) were purchased from Supelco (Bellefonte, PA) for use as standards. Solvents used were of analytical grade and were distilled before use. *Lipid extraction.* The total lipids (TLs) were extracted from perilla cultivars with diethyl ether for 8 h in a Soxhlet apparatus. The solvent was removed under reduced pressure, and the percentage of total lipids was calculated.

Physicochemical characteristics and tocopherol determination. The refractive index, iodine value, saponification value and unsaponifiable matter of the TLs were determined by American Oil Chemists' Society methods Cc 7-25, Cd 1-25, Cd 30-25 and Ca 6a-40, respectively (2). Tocopherols in perilla oils were analyzed by normal-phase high-performance liquid chromatography (Waters Associates, Milford, MA) as described by Carpenter (3). D- α -Tocopherol was used for a standard curve as well as an external standard.

Lipid classes analysis. The (TLs) were fractionated into NL, GL and PL on a silicic acid column (4) with chloroform, acetone and methanol, respectively. NL were estimated gravimetrically; GL and PL were quantitated by total sugar estimation (5) and phosphorus estimation (6), respectively.

Lipid classes of the NL, GL and PL were separated by thin-layer chromatography (TLC) on silica gel plates. For separation of NL, silica gel G plates (0.25 mm) and a solvent system of n-hexane/diethyl ether/acetic acid (90:10:1, vol/vol/vol) was used. Individual components of NL were identified by comparison with known standards and quantitated by photodensitometry (7). For separation of GL and PL, silica gel plates (0.50 mm) and the following solvent systems were used: GL, chloroform/methanol/water (75:25:4, vol/vol/vol); and PL, chloroform/methanol/water/28% aqueous ammonia (65:35:4:0.2, by vol). Individual components of GL and PL were identified by comparison with authentic standards and by using specific sprays, such as perchloric acid for GL (8) and ninhydrin, Dragendorff's reagent or molybdenum reagent for PL (9). Individual comonents of GL and PL on preparative TLC were determined by estimation of sugar (5) and phosphorus (6) contents, respectively.

Fatty acid analysis. Fatty acid compositions of the TLs and of the three fractions were determined after conversion to FAME as described by Morrison and Smith (10). The FAME were analyzed in a Hewlett-Packard (Palo Alto, CA) 5890A gas chromatograph with flame-ionization detector. A glass column ($2 \text{ m} \times 2 \text{ mm i.d.}$), packed with 10% (w/w) diethyleneglycol succinate on 100-200 mesh Chromosorb WHP, was used for methyl ester separation. The column oven temperature was 190°C, the injection temperature was maintained at 210°C and the nitrogen carrier gas flow rate was 40 mL/min. The emerging peaks were identified by comparing retention times with those of a standard mixture of known FAME. The peak area and relative percentage of FAME were obtained with a Hewlett-Packard model 3390A integrator.

RESULTS AND DISCUSSION

Physicochemical properties and tocopherol contents of TL in perilla seed are shown in Table 1. The TL contents of the five perilla seed cultivars varied from 38.6 to 47.8%

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TABLE 1

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Cultivar	Total lipids	Refractive index (N _D ²⁵)	Iodine value	Saponification value	Unsaponifiable matter (%)	Isomer of tocopherol (mg/100 g)				
	$(wt)^{\delta}$					α-	β-	γ-	6-	Tota
Suwon 8	39.5	1.4760	192.0	192.7	1.3	1.4	c	50.8	2.9	55.1
Suwon 10	43.3	1.4771	193.7	193.9	1.5	0.9		54.5	4.3	59.7
Suwon 21	45.6	1.4784	196.3	194.9	1.6	0.6		46.3	2.2	49.1
Suwon 24	38.6	1.4775	195.3	192.9	1.8	0.7	_	48.8	3.0	52.5
Yaebsil	47.8	1.4780	193.8	197.7	1.4	1.5		62.8	3.3	67.6

Physicochemical Characteristics and Tocopherol Content of Total Lipids in Perilla Seeds^a

^aAll values are means of three replicate analyses.

^bPercentages of the seed on dry weight basis.

^cNot detected.

(w/w) on a dry weight basis. The lipid content of Suwon 24 was the lowest, and that of Yaebsil was the highest among the five cultivars in this study. Selected physicochemical properties of the lipid extracted from perilla seeds are as follows: refractive index at 25°C, 1.4760-1.4784; iodine value (Wijs), 192.0-196.3; saponification value, 192.7-197.7; unsaponifiable matter, 1.3-1.8%. The total tocopherol contents of TLs in perilla seeds varied between 49.1 and 67.6 mg/100 g oil. The major tocopherol in perilla oil was in the γ -form at 92% of total tocopherol. The a- and o-tocopherols were found at relatively low concentrations, whereas the β -form was not detected in any of the five perilla seed oils (Table 1). Major lipid classes of perilla seed are shown in Table 2. TL composition in perilla was 91.2-93.9% NL, 3.9-5.8% GL and 2.0-3.0% PL.

The major fatty acids of the TL fraction in perilla oil were linolenic acid (61.1-64.0%), linoleic acid (14.3-17.0%) and oleic acid (13.2-14.9%). In particular, perilla oil contained high levels of linolenic and linoleic acids, similar to linseed oil (11). Therefore, perilla oil may be a good source of essential fatty acids. Recently, many dietitians have been concerned about an importance of balanced fatty acid intake in terms of ω -6 and ω -3 fatty acid. From this point of view, this oil may be used to optimize the ratio of ω -6 and ω -3 fatty acids by blending with other oils. On the other hand, it may be easily oxidized during processing and storage because of its high unsaturated fatty acid content. The patterns of major fatty acid composition of TLs among the five perilla seed cultivars were fairly similar (Table 3). The fatty acid profile of NL largely

reflected that of TL, whereas GL and PL fractions had the highest content of palmitic, stearic and linoleic acids, as compared with TL and NL. Capric acid was detected in both GL and PL of Suwon 10, Suwon 21 and Yaebsil but was not detected in GL of Suwon 8, or in GL and PL of Suwon 24. Myristic acid was detected in GL of four perilla seed cultivars, except for Suwon 8 (Table 3).

otal

The NLs fraction contained 88.1-91.0% triacylglycerols, 4.1-6.2% sterol esters and hydrocarbons, 1.9-2.7% free sterols and small amounts of partial glycerides and free fatty acids (Table 4). Among the GL, esterified sterylglycoside (48.9-53.2%) and sterylglycoside (22.1-25.4%) were the most abundant. Monogalactosyldiacylglycerol and digalactosyldiacylglycerol were present in small quantities. The PL fraction was resolved into six components by TLC. The major PL were phosphatidylethanolamine (50.4-57.1%), phosphatidylcholine (17.6-20.6%) and phosphatidic acid (13.6-19.9%). Lysophosphatidylcholine, phosphatidylserine and phosphatidylinositol were present in small quantities. The individual composition patterns in NL, GL and PL were not significantly different among the five perilla seed cultivars.

Little has been published about lipid composition and physicochemical properties of perilla seed. Results of this study have provided basic information about lipid classes and fatty acid composition of perilla seed. However, further research is required to find out how to enhance oxidative stability of perilla oil, and to explore the relationship between lipid composition and flavor characteristics of perilla oil.

TABLE 2

Major Lipid Classes of Perilla Seed^a

	Neutral lipids		Glycolipids		Phospholipids	
Cultivar	\mathbf{wt}^b	% ^c	$\overline{\mathbf{wt}^b}$	% ^c	\mathbf{wt}^{b}	% ^c
Suwon 8	36.9	93.5	1.5	3.9	1.1	2.7
Suwon 10	40.7	93.9	1.8	4.2	0.8	2.0
Suwon 21	42.7	93.7	2.0	4.4	0.9	2.0
Suwon 24	35.2	91.2	2.2	5.8	1.2	3.0
Yaebsil	44.2	92.5	2.5	5.2	1.1	2.3

^aAll values are means of three replicate analyses.

^bPercentages of the seed on dry weight basis.

^cPercentages represent the fraction of a given lipid class with respect to total lipid content within a cultivar.

TABLE 3	3
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Fatty Acid Composition of Total Lipids and Major Lipid Classes in Perilla Seeds^a

	$\begin{array}{c} \text{Lipid} \\ \text{class}^{b} \end{array}$	Fatty acid composition (%)						
Cultivar		10:0	14:0	16:0	18:0	18:1	18:2	18:3
	TL	_	_	6.7	1.5	13.6	17.0	61.1
Suwon 8	NL	_	_	6.3	1.6	13.3	16.0	62.8
	GL	_	_	8.0	2.7	14.6	18.6	56.1
	\mathbf{PL}	3.2	—	7.7	1.5	14.1	15.3	58.2
	\mathbf{TL}	_	_	6.4	1.6	13.2	16.0	62.8
Suwon 10	NL	_	_	6.9	1.5	13.0	15.8	62.9
	GL	6.1	1.6	14.6	4.4	13.3	17.9	42.1
	\mathbf{PL}	18.3		13.0	2.9	8.5	25.7	31.6
	\mathbf{TL}	_	_	6.3	1.6	13.7	14.6	63.8
Suwon 21	NL	_		6.5	1.7	14.1	14.6	63.2
	\mathbf{GL}	1.0	15.2	0.5	3.9	14.0	16.4	49.1
	\mathbf{PL}	7.0	—	15.9	3.4	6.9	25.4	41.5
	\mathbf{TL}	_	_	6.5	1.7	14.9	15.7	61.2
Suwon 24	NL	-	_	6.7	1.6	14.9	14.7	62.1
	GL	_	2.0	12.8	3.5	13.7	18.6	49.3
	\mathbf{PL}	_		15.2	3.2	8.5	29.3	43.8
	TL	_		6.3	1.6	13.8	14.3	64.0
Yaebsil	NL	_		6.4	1.4	13.7	14.4	64.0
	\mathbf{GL}	14.1	1.4	13.8	3.1	13.0	14.3	40.2
	\mathbf{PL}	15.7	-	14.0	2.4	8.3	23.8	35.9

^aAll values are means of three replicate analyses.

^bTL, total lipids; NL, neutral lipids; GL, glycolipids; PL, phospholipids.

TABLE 4

Lipid class	Suwon 8	Suwon 10	Suwon 21	Suwon 24	Yaebsil
Neutral lipids ^b					
SE, HC	4.1	6.2	5.7	7.5	5.6
TG	91.0	89.1	88.7	88.1	88.2
FFA	0.3	0.4	0.7	0.3	0.8
FS	1.9	2.5	2.5	2.6	2.7
DG	0.8	0.3	0.9	0.4	1.2
MG	1.8	1.5	1.4	1.0	1.4
Glycolipids ^c					
SG	25.4	23.3	22.1	23.2	24.4
DGDG	7.9	9.2	8.1	8.0	9.4
ESG	50.8	48.9	54.3	53.2	51.4
MGDG	15.8	18.6	15.4	15.6	14.7
Phospholipids ^d					
LPĆ -	4.0	3.6	3.6	4.0	2.9
PS, PI	4.8	6.1	6.1	6.5	6.6
PC	20.6	17.6	17.9	19.4	20.2
PE	54.2	55.4	57.1	56.6	50.4
PA	16.3	17.4	15.3	13.6	19.9

^aAll values are percentages of each lipid class. ^bSE, sterol esters; HC, hydrocarbons; TG, triacylglycerols; FFA, free fatty acids; FS, free sterols; DG, diacylglycerols; MG, monoacylglycerols. ^cSG, sterylglycoside; DGDG, digalactosyldiacylglycerol; ESG, esterified sterylglycoside;

MGDG, monogalactosyldiacylglycerol.

^dLPC, lysophosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PA, phosphatidic acid.

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