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Metabolite Profiling Based on Lipophilic Compounds for Quality Assessment of Perilla (*Perilla frutescens*) Cultivars

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ABSTRACT: Lipophilic compounds from Korean perilla (*Perilla frutescens*) seeds were characterized to determine the diversity among their phytochemicals and to analyze relationships between their contents. Twenty-four metabolites consisting of policosanol, phytosterol, tocopherol, and fatty acids were identified. The metabolite profiles were subjected to data mining processes, including principal component analysis (PCA), partial least-squares discriminate analysis (PLS-DA), and Pearson's correlation analysis. PLS-DA could distinguish between all cultivars except between Daesil and Daeyeup cultivars. Linolenic acid contents were positively correlated with β-sitosterol ($r = 0.8367$, $P < 0.0001$) and γ-tocopherol contents ($r = 0.7201$, $P < 0.001$) among all perilla grains. The Daesil and Daeyeup cultivars appear to be good candidates for future breeding programs because they have simultaneously high linolenic acid, phytosterol, and tocopherol levels. These results demonstrate the use of metabolite profiling as a tool for assessing the quality of food.

KEYWORDS: *perilla, fatty acid, tocopherol, phytosterol, policosanol, metabolite profiling, principal component analysis*

INTRODUCTION

Perilla (*Perilla frutescens*) seeds are a traditional source of oils produced in Korea, India, China, and other Asian countries. Perilla seeds are rich in tocopherol,[1] phytosterol,[2] and other natural nutrients such as policosanol.[3] Tocopherol is frequently used in foods as an antioxidant. Dietary tocopherol, especially α-tocopherol, can prevent oxidative stress in vivo.[4] Furthermore, tocopherol was found to reduce viral load in patients with HIV,[5] to moisturize the skin,[6] and to have positive effects in the treatment of Parkinson syndrome.[7] Phytosterol has also been shown to reduce serum total cholesterol,[8,9] reduce low-density lipoprotein (LDL) cholesterol levels, and increase high-density lipoprotein (HDL) cholesterol levels in the blood.[10] Octacosanol, one of the most abundant alcohols in policosanols, has cytoprotective effects and is taken as an alternative to aspirin in patients suffering from gastric irritation.[11] These lipids are becoming more popular because of their positive health effects. Therefore, the perilla seed is an important dietary component, and its natural lipophilic components warrant evaluation.

Perilla seed oil contains high levels of linolenic acid.[12] Adhikari et al. reported that policosanols in the perilla seed are composed of 67−68% octacosanol, 16−17% hexacosanol, and 6−9% triacontanol.[3] Tatematsu et al. reported a nutritional evaluation, and the sterol composition was examined by Perilla-Lard.[2] Despite the nutritional benefits of perilla seeds, few papers have described their biofortification to improve nutritional quality through conventional breeding.[3,13] Investigating the diversity of phytochemicals among perilla varieties is required to allow for enrichment of these components through breeding. However, to the best of our knowledge, a comprehensive study regarding tocopherol, phytosterol, and policosanol levels in perilla seeds from different varieties has not been reported.

This study evaluated the lipophilic composition such as fatty acids, tocopherols, phytosterols, and policosanols in perilla grains and analyzed the relationship between their contents. Metabolomics allows for sample classification of diverse biological status, origin, or quality using chemometrics such as principal component analysis (PCA) and partial least-squares discriminate analysis (PLS-DA). PCA and PLS-DA are the most common chemometric tools for extracting and rationalizing information from any multivariate description of a biological system.[14] Recently, Tianniam et al. examined differences between *Angelica acutiloba* qualities using multivariate pattern recognition.[15] Thus, we applied PCA and PLS-DA to examine and compare the quality of perilla cultivars. Our study's findings could provide perilla breeders, and eventually commercial perilla growers, new opportunities to promote the production of perilla with enhanced levels of bioactive compounds.

MATERIALS AND METHODS

Samples and Chemicals. Seven cultivars of perilla (Baekkwang, Daesil, Daeyeup, Hwahong, Ipdlkkae1, Okdong, and Saeyeopcil) were used in this study. All perilla grains were harvested at the Bioherb Research Institute, Kangwon National University, Republic of Korea, in 2010. The perilla grains were kept at −80 °C before extraction. Eicosanol, heneicosanol, tricosanol, tetracosanol, hexacosanol, heptacosanol, octacosanol, triacontanol, 5α-cholestan-3β-ol, campesterol, stigmasterol, β-sitosterol, 5α-cholestane, pyridine, ascorbic acid, *N*-methyl-*N*-trimethylsilyltrifluoroactamide (MSTFA), and docosanol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Tocopherols were purchased from Merck (Darmstadt, Germany). All other chemicals used in this study were of reagent grade unless otherwise stated.

Extraction and Analysis of Lipophilic Compounds. Extraction, separation, and measurement of phytosterol and tocopherol by gas chromatography−time-of-flight mass spectrometry (GC-TOFMS) were performed as described previously.[16] Phytosterols and tocopherols

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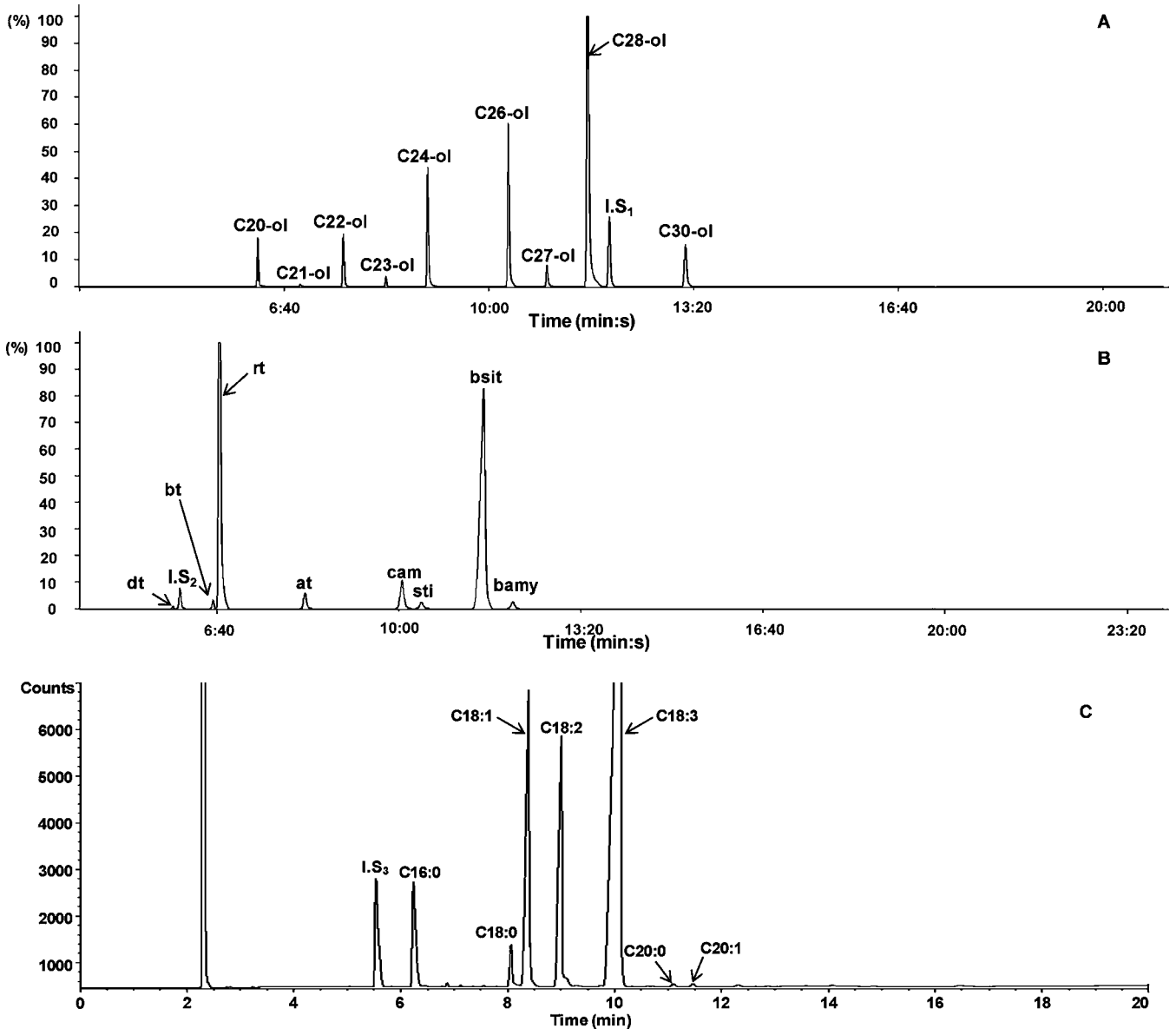

Figure 1. MS (selected-ion) chromatograms of policosanols (A) and phytosterols and tocopherols (B) as TMS derivatives and GC chromatogram (C) of fatty acid as ester derivatives extracted from a perilla sample (cv. Daesil). The peaks correspond to the following: C20-ol, eicosanol; C21-ol, heneicosanol; C22-ol, docosanol; C23-ol, tricosanol; C24-ol, tetracosanol; C26-ol, hexacosanol; C27-ol, heptacosanol; C28-ol, octacosanol; C30-ol, triacontanol; dt, δ-tocopherol; bt, β-tocopherol; rt, γ-tocopherol; at, α-tocopherol; cam, campesterol; sti, stigmasterol; bsit, β-sitosterol; bamy, β-amyrin; C16:0, palmitic acid; C18:0, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; C18:3, linolenic acid; C20:0, arachidic acid; C20:1, eicosenoic acid; I.S$_1$, β-cholestanol; I.S$_2$, 5α-cholestane; I.S$_3$, pentadecanoic acid.

were released from the powdered perilla samples (0.05 g) by adding 3 mL of ethanol containing 0.1% ascorbic acid (w/v) and 0.05 mL of 5α-cholestane (10 μg/mL) as the internal standard (IS), mixed by vortexing for 20 s, and placed in a water bath at 85 °C for 5 min. After removal from the water bath, 120 μL of potassium hydroxide (80%, w/v) was added, and the samples were vortexed for 20 s and returned to the water bath for 10 min. After saponification, samples were immediately placed on ice, and deionized water (1.5 mL) was added. Hexane (1.5 mL) was then added to each sample and vortexed for 20 s, followed by centrifugation (1200g, 5 min). The upper layer was pipetted into a separate tube, and the pellet was re-extracted using hexane. The hexane fraction was dried in a centrifugal concentrator (CVE-2000; Eyela, Tokyo, Japan). For derivatization, 30 μL of MSTFA with 30 μL of pyridine was added and incubated at 60 °C for 30 min at a mixing frequency of 1200 rpm using a thermomixer comfort (model 5355; Eppendorf AG, Hamburg, Germany). GC-TOFMS was performed using a gas chromatograph (7890A; Agilent, Atlanta, GA, USA) coupled to a Pegasus HT TOF mass spectrometer (LECO, St. Joseph, MI, USA). A derivatized sample (1 μL) was separated on a 30 m × 0.25 mm i.d. fused-silica capillary column coated with 0.25 μm CP-SIL 8 CB low bleed (Varian Inc., Palo Alto, CA, USA). The injector temperature was 290 °C, the split ratio was set at 1:10, and the helium gas flow rate through the column was 1.0 mL/min. The temperature program was set at 250 °C, followed by a 10 °C/min oven temperature ramp to 290 °C and a 20 min heating at 290 °C. The transfer line and the ion source temperatures were 280 and 230 °C, respectively. The scanned mass range was m/z 50−600, and the detector voltage was set at 1700 V. For quantification purposes, seven calibration samples in hexane were prepared by mixing increasing amounts (equivalent to 0.03, 0.05, 0.10, 0.25, 0.50, 1.00, and 5.00 μg) of tocopherol and phytosterol standards and a fixed amount (0.50 μg each) of the IS. As described above, each sample was subjected to saponification, followed by re-extraction and trimethylsilyl (TMS) etherification. The quantitative calculation was based on the corrected peak area ratios relative to the peak area of the IS.

Table 1. Detected Gas Chromatographic and Mass Spectrometric Data of Trimethylsilyl Derivatives of Policosanols, Phytosterols, and Tocopherols Identified by GC-TOFMS in a Perilla Sample

abbreviation	compound	RT[a]	RRT[b]	quantification ion[c]
	Policosanols			
C20-ol	eicosanol	6.14	0.530	355
C21-ol	heneicosanol	6.56	0.567	369
C22-ol	docosanol	7.38	0.638	383
C23-ol	tricosanol	8.19	0.708	397
C24-ol	tetracosanol	9.00	0.778	411
C26-ol	hexacosanol	10.19	0.881	439
C27-ol	heptacosanol	10.57	0.913	453
C28-ol	octacosanol	11.36	0.981	467
C30-ol	triacontanol	13.11	1.133	495
	Phytosterols and Tocopherols			
dt	δ-tocopherol	5.52	0.920	208
bt	β-tocopherol	6.36	1.060	222
rt	γ-tocopherol	6.43	1.072	223
at	α-tocopherol	8.17	1.361	237
cam	campesterol	10.04	1.673	343
sti	stigmasterol	10.25	1.708	394
bsit	β-sitosterol	11.34	1.890	357
bamy	β-amyrin	12.06	2.010	218

[a]Retention time (min). [b]Relative retention time (retention time of the analyte/retention time of the internal standard). [c]Specific mass ion used for quantification.

The linear equations and regression coefficients for α-tocopherol, β-tocopherol, γ-tocopherol, δ-tocopherol, β-amyrin, β-sitosterol, campesterol, and stigmasterol were $y = 2.4217x + 0.0171$, with $r = 0.9990$; $y = 3.2005x + 0.1807$, with $r = 0.9971$; $y = 3.2419x + 0.0515$, with $r = 0.9989$; $y = 2.7849x + 0.0441$, with $r = 0.9988$; $y = 5.0443x + 0.8634$, with $r = 0.9994$; $y = 0.2582x + 0.0097$, with $r = 0.9997$; $y = 0.3518x + 0.0106$, with $r = 0.9995$; and $y = 0.1484x + 0.0137$, with $r = 0.9998$, respectively.

Extraction of policosanols was the same as described above, except for using β-cholestanol (2.00 μg) as the IS. GC-TOFMS analysis was performed as described above, except the temperature program was set at 200 °C, followed by a 10 °C/min oven temperature ramp to 310 °C and a 10 min heating at 310 °C. For quantification purposes, calibration curves were drawn by plotting at 10 different concentrations of policosanol standards according to the peak area ratios with β-cholestanol. The responses were linear in the following ranges: 0.008−5.000 μg ($y = 0.9735x + 0.0029$, with $r = 0.9999$); 0.008−5.000 μg ($y = 0.8418x + 0.0100$, with $r = 0.9997$); 0.008−5.000 μg ($y = 0.7504x + 0.0110$, with $r = 0.9995$); 0.008−5.000 μg ($y = 0.5732x + 0.0134$, with $r = 0.9995$); 0.008−5.000 μg ($y = 0.5518x + 0.0186$, with $r = 0.9991$); 0.008−5.000 μg ($y = 0.5919x + 0.0188$, with $r = 0.9995$); 0.008−5.000 μg ($y = 0.4921x + 0.0122$, with $r = 0.9994$); 0.008−5.000 μg ($y = 0.3920x + 0.0091$, with $r = 0.9995$); and 0.019−5.000 μg ($y = 0.3200x + 0.0107$, with $r = 0.9992$), for eicosanol, heneicosanol, docosanol, tricosanol, tetracosanol, hexacosanol, heptacosanol, octacosanol, and triacontanol, respectively.

The individual fatty acid contents were determined after lipid extraction and saponification with 0.5 M sodium hydroxide in methanol.[17] Pentadecanoic acid was used as the IS. The saponification mixture was methylated with 14% boron trifluoride/methanol, and the

Table 2. Content (Milligrams per 100 g) of Lipophilic Compounds in Seven Perilla Cultivars[a]

	Baekkwang	Daesil	Daeyeup	Hwahong	Ipdlkkae1	Okdong	Saeyeopcil
policosanols							
C20-ol	0.49 ± 0.05b	0.65 ± 0.22b	0.69 ± 0.09b	0.56 ± 0.10b	1.72 ± 0.12a	0.60 ± 0.09b	0.63 ± 0.17b
C21-ol	0.04 ± 0.00b	0.05 ± 0.01b	0.05 ± 0.00b	0.05 ± 0.00b	0.08 ± 0.00a	0.05 ± 0.00b	0.05 ± 0.01b
C22-ol	0.69 ± 0.02b	0.93 ± 0.29b	0.89 ± 0.14b	0.78 ± 0.14b	2.04 ± 0.25a	0.80 ± 0.11b	0.81 ± 0.21b
C23-ol	0.29 ± 0.01b	0.25 ± 0.07b	0.23 ± 0.04b	0.32 ± 0.05b	0.67 ± 0.08a	0.33 ± 0.05b	0.33 ± 0.07b
C24-ol	2.31 ± 0.02b	2.96 ± 0.82ab	2.44 ± 0.32b	3.90 ± 0.71a	3.97 ± 0.43a	3.20 ± 0.31ab	3.44 ± 0.96ab
C26-ol	2.92 ± 0.01c	3.49 ± 0.56bc	5.04 ± 0.19a	3.13 ± 0.31c	4.16 ± 0.21b	3.53 ± 0.47bc	3.07 ± 0.63c
C27-ol	0.51 ± 0.01b	0.57 ± 0.10ab	0.71 ± 0.03a	0.55 ± 0.06ab	0.71 ± 0.04a	0.62 ± 0.11ab	0.56 ± 0.15ab
C28-ol	14.18 ± 0.39b	15.01 ± 1.54ab	17.80 ± 1.35a	13.90 ± 1.16b	15.46 ± 0.52ab	16.87 ± 1.74ab	13.95 ± 3.10b
C30-ol	2.04 ± 0.07 cd	2.01 ± 0.33 cd	1.58 ± 0.18d	2.16 ± 0.14bc	2.06 ± 0.09 cd	3.07 ± 0.14a	2.58 ± 0.52b
total	23.48 ± 0.52	25.92 ± 3.43	29.43 ± 1.21	25.35 ± 2.64	30.87 ± 1.09	29.06 ± 2.51	25.41 ± 5.42
tocopherols							
at	0.91 ± 0.13a	1.01 ± 0.12a	1.00 ± 0.05a	0.92 ± 0.08a	0.62 ± 0.05b	0.89 ± 0.08a	0.90 ± 0.16 a
bt	0.13 ± 0.04a	0.16 ± 0.01a	0.16 ± 0.01a	0.16 ± 0.01a	0.15 ± 0.02a	0.16 ± 0.00a	0.17 ± 0.01a
rt	15.32 ± 0.79a	14.53 ± 0.96a	15.26 ± 1.74a	13.54 ± 2.07ab	11.50 ± 1.64b	13.56 ± 1.68ab	13.33 ± 1.47ab
dt	0.06 ± 0.02b	0.06 ± 0.03b	0.08 ± 0.01b	0.01 ± 0.00c	0.17 ± 0.01a	0.08 ± 0.01b	0.09 ± 0.04b
total	16.43 ± 0.89	15.75 ± 1.05	16.50 ± 1.79	14.64 ± 2.14	12.44 ± 1.70	14.70 ± 1.66	14.48 ± 1.53
phytosterols							
bamy	0.52 ± 0.03bc	0.54 ± 0.02bc	0.79 ± 0.04a	0.47 ± 0.03 cd	0.38 ± 0.10d	0.61 ± 0.05b	0.54 ± 0.06bc
bsit	352.62 ± 8.73ab	385.44 ± 25.09a	385.88 ± 20.41a	324.55 ± 26.24bc	285.09 ± 29.73c	310.03 ± 36.77bc	305.95 ± 19.17bc
cam	10.53 ± 0.21ab	10.95 ± 0.24a	9.88 ± 0.27bc	9.53 ± 0.33c	10.83 ± 0.75a	9.59 ± 0.57c	9.69 ± 0.44c
sti	4.18 ± 0.10b	5.72 ± 0.02a	4.51 ± 0.98ab	3.68 ± 0.78b	4.54 ± 0.31ab	4.60 ± 0.93ab	4.94 ± 0.74ab
total	367.86 ± 8.57	402.66 ± 25.36	401.07 ± 20.71	338.23 ± 27.30	300.83 ± 30.88	324.83 ± 38.11	321.12 ± 20.26
fatty acids							
C16:0	2.65 ± 0.21bc	3.61 ± 0.28a	2.94 ± 0.34b	2.63 ± 0.20bc	2.07 ± 0.18d	2.96 ± 0.08b	2.38 ± 0.13 cd
C18:0	0.78 ± 0.06bc	0.96 ± 0.11ab	0.96 ± 0.11ab	0.80 ± 0.07bc	0.65 ± 0.07c	0.99 ± 0.11a	0.74 ± 0.11c
C18:1	6.77 ± 0.65d	8.69 ± 0.64ab	7.37 ± 0.65bcd	8.29 ± 0.67abc	6.29 ± 0.43d	9.28 ± 1.07a	6.93 ± 0.96 cd
C18:2	6.95 ± 0.63bcd	8.19 ± 0.49ab	7.23 ± 0.86bc	5.46 ± 0.60de	5.14 ± 0.51e	9.53 ± 1.57a	6.60 ± 0.50cde
C18:3	34.48 ± 2.50bcd	42.07 ± 2.13a	39.03 ± 4.53ab	32.80 ± 2.52 cd	24.05 ± 1.69e	35.87 ± 3.41bc	29.83 ± 1.11d
C20:0	0.05 ± 0.01b	0.07 ± 0.01a	0.06 ± 0.01ab	0.05 ± 0.01b	0.06 ± 0.01ab	0.07 ± 0.01a	0.05 ± 0.00b
C20:1	0.06 ± 0.01b	0.08 ± 0.01a	0.06 ± 0.01b	0.06 ± 0.01b	0.05 ± 0.01b	0.08 ± 0.01a	0.06 ± 0.00b
total	51.75 ± 4.07	63.66 ± 3.68	57.65 ± 6.51	50.12 ± 4.08	38.31 ± 2.88	58.78 ± 6.25	46.60 ± 2.81

[a]Each value is the mean of three replications ± standard deviation. Different letters represent significant ($P < 0.05$) differences between means according to ANOVA combined with Duncan's multiple-range test.

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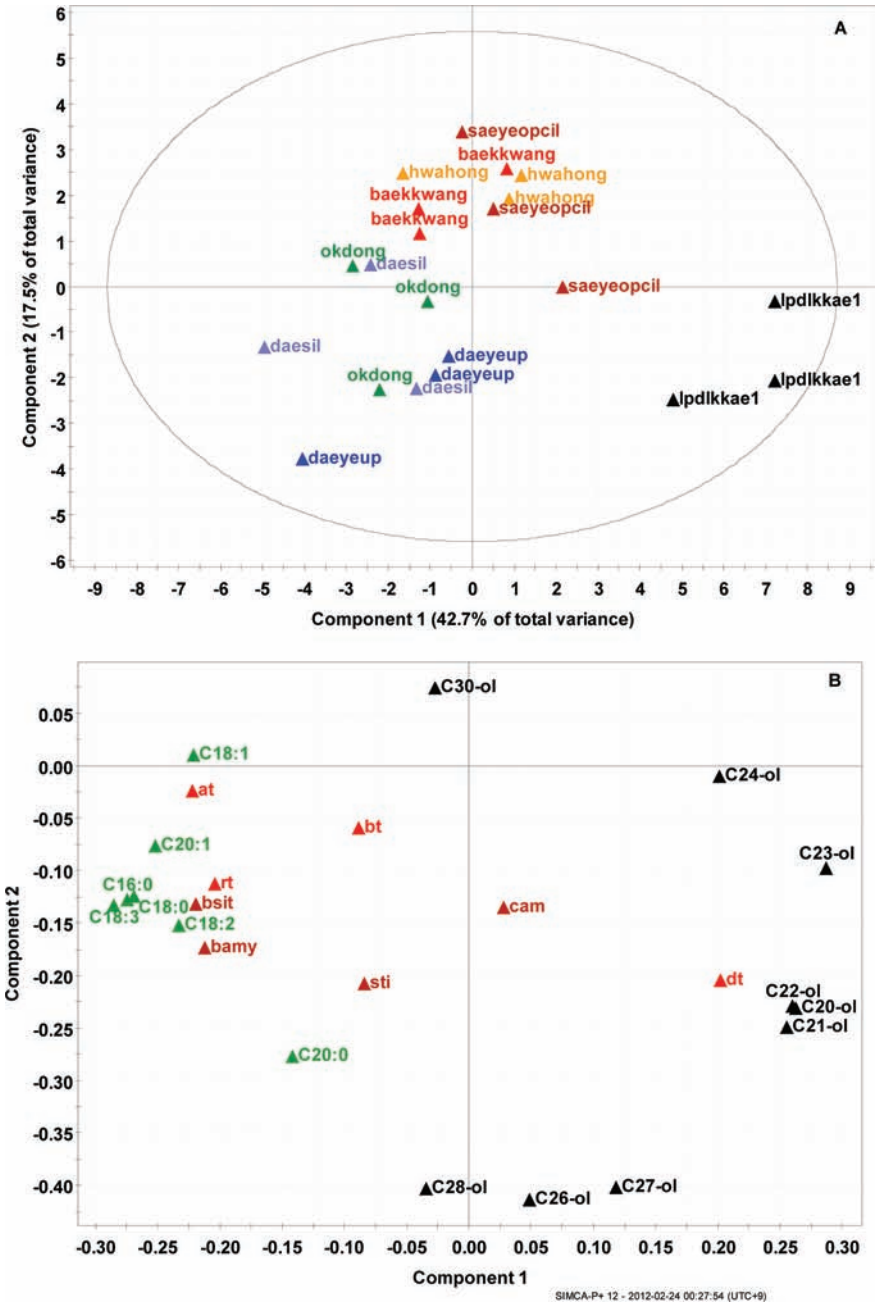

Figure 2. Scores (A) and loading (B) plots of principal components 1 and 2 of the PCA results obtained from lipophilic compound data on seven perilla cultivars. C20-ol, eicosanol; C21-ol, heneicosanol; C22-ol, docosanol; C23-ol, tricosanol; C24-ol, tetracosanol; C26-ol, hexacosanol; C27-ol, heptacosanol; C28-ol, octacosanol; C30-ol, triacontanol; dt, δ-tocopherol; bt, β-tocopherol; rt, γ-tocopherol; at, α-tocopherol; cam, campesterol; sti, stigmasterol; bsit, β-sitosterol; bamy, β-amyrin; C16:0, palmitic acid; C18:0, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; C18:3, linolenic acid; C20:0, arachidic acid; C20:1, eicosenoic acid.

resulting methyl esters were extracted with pentane. The methyl esters of the fatty acids were injected into the GC using an autosampler (Agilent 6890). A Hewlett-Packard 5980 gas chromatograph equipped with a flame ionization detector (Agilent) and DB-WAX capillary column (30 m × 0.25 mm i.d.; Agilent) was used. The oven temperature was raised from 130 to 190 °C at a constant rate of 25 °C/min and maintained at 190 °C for 5 min, followed by a 3 °C/min oven temperature ramp to 230 °C and a 3 min heating at 230 °C. The injector and detector temperatures were kept at 250 °C. Fatty acids were identified by comparison with standard mixtures of fatty acid methyl esters and the results calculated using response factors derived from chromatograph standard of known position. The quantitative calculation was based on the corrected peak area ratios relative to the peak area of IS.

Statistical Analysis. All analyses were performed at least in triplicate. Experimental data were analyzed by an analysis of variance (ANOVA) using SAS 9.2 (SAS Institute, Cary, NC, USA), and the significant differences among means were determined by Duncan's multiple-range test. Quantification levels of metabolites were subjected to PCA and PLS-DA (SIMCA-P version 12.0; Umetrics, Umeå, Sweden) to evaluate the relationships in terms of similarity or dissimilarity among groups of multivariate data. The PCA and PLS-DA output consisted of score plots to visualize the contrast between different samples and loading plots to explain the cluster separation. Pearson's correlation analysis was performed using the SAS 9.2 software package. The correlation analysis was performed among the contents of 24 metabolites.

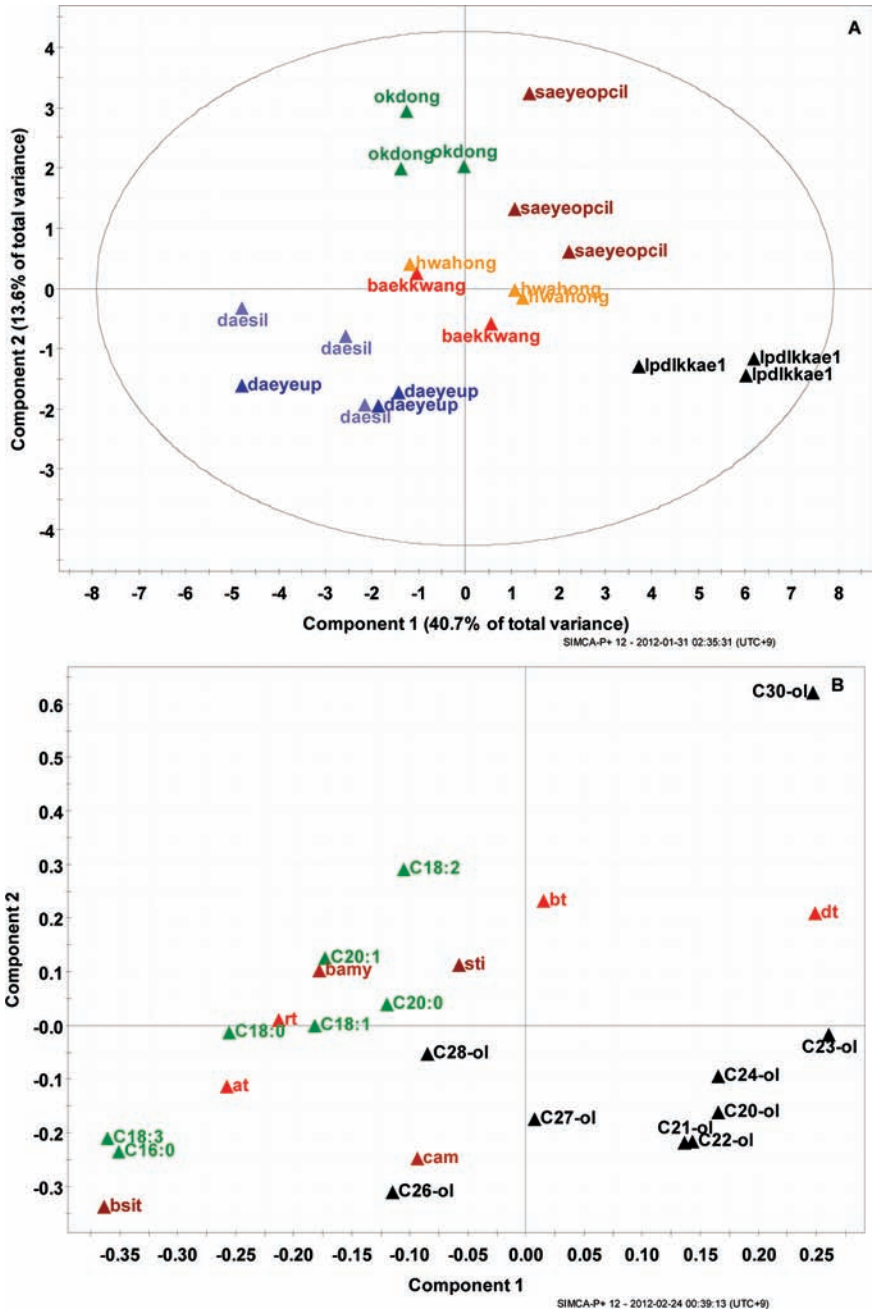

Figure 3. Scores (A) and loading (B) plots of principal components 1 and 2 of the PLS-DA results obtained from lipophilic compound data on seven perilla cultivars. C20-ol, eicosanol; C21-ol, heneicosanol; C22-ol, docosanol; C23-ol, tricosanol; C24-ol, tetracosanol; C26-ol, hexacosanol; C27-ol, heptacosanol; C28-ol, octacosanol; C30-ol, triacontanol; dt, δ-tocopherol; bt, β-tocopherol; rt, γ-tocopherol; at, α-tocopherol; cam, campesterol; sti, stigmasterol; bsit, β-sitosterol; bamy, β-amyrin; C16:0, palmitic acid; C18:0, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; C18:3, linolenic acid; C20:0, arachidic acid; C20:1, eicosenoic acid.

RESULTS AND DISCUSSION

In this study, lipophilic compounds such as policosanol, phytosterol, and tocopherol from perilla seeds were identified by GC-TOFMS, and the fatty acid composition was identified by GC−flame ionization detector (FID) (Figure 1). Twenty-four types of lipophilic compounds, including 9 policosanols, 4 phytosterols, 4 tocopherols, and 7 fatty acids, were detected in all cultivars. ChromaTOF software was used to support peak findings prior to quantitative analysis and for automated deconvolution of reference mass spectra. Quantification was performed using selected ions as described in Table 1. Precursor ions and product ions in the mass spectra of the tocopherol and phytosterol agreed with those in the literature.[18,19] The mass spectra of policosanol TMS derivatives showed that the molecular ion $[M - 15]^+$ was generally dominant. In total, 24 lipophilic profiles in perilla seeds were identified.

Most vegetable oils are good sources of linoleic acid, but very few vegetable oils contribute significant amounts of linolenic acid. Among them, perilla seed oil has the highest linolenic acid levels.[12] In this study, the linolenic acid content in the analyzed samples ranged from 60.3 to 68.0% of the total fatty acids (Table 2). These results were similar to those reported in previous studies on perilla oil in which the linolenic acid content ranged from 61.1 to 64.0%.[13] As reported earlier,[1] γ-tocopherol

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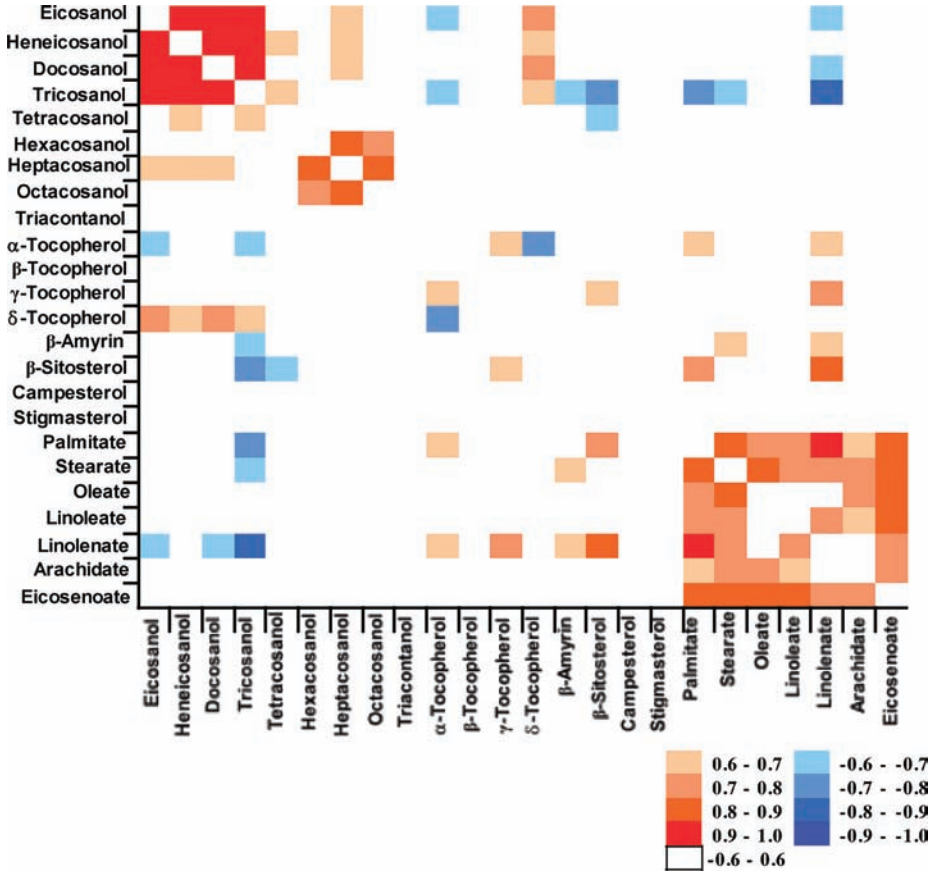

Figure 4. Correlation matrix of lipophilic compounds from seven perilla cultivars. Each square indicates Pearson's correlation coefficient of a pair of compounds, and the value of the correlation coefficient is represented by the intensity of blue or red colors, as indicated on the color scale.

(11.5−15.3 mg/100 g) was the major tocopherol in perilla seeds (Table 2).

Traditionally, food component analysis involved identifying food constituents into very broad categories such as proteins, fats, carbohydrates, fiber, vitamins, trace elements, solids, and/or ash. However, with the advent of metabolomics, metabolite profiling combined with chemometrics has been employed to direct breeding strategies to enhance specific desired balances of food components in fresh food, which have been identified as being more optimal.[20] PCA and PLS-DA were used to arrange unsupervised and supervised large, complex data sets, respectively. As a clustering technique, PCA or PLS-DA is most commonly used to identify how one sample is different from another, which variables contribute most to this difference, and whether those variables are correlated or uncorrelated.[14,20] The data obtained for the 24 lipophilic compounds detected were subjected to PCA to outline the lipophilic profile differences among cultivars and to assess the overall experimental variation (Figure 2). The results were attained by plotting the principal component scores. The first component of the PCA explained approximately 42.7% of the variation, and the second component explained an additional 17.5% of the variation. The first component resolved the measured composition profiles of the lpdlkkae1 cultivar and other cultivars. Identifying compounds with the greatest variance within a population and determining closely related compounds is also possible using PCA.[20] To further investigate contributors to the components, we compared the factor loadings in components 1 and 2. In component 1, the variation was mainly attributed to policosanol and fatty acid, of which the corresponding loading was positive for all policosanols except octacosanol and triacontanol and negative for all fatty acids, α-tocopherol, γ-tocopherol, β-amyrin, and β-sitosterol. However, PCA could not distinguish among Daesil, Daeyeup, and Okdong cultivars or among Baekkwang, Hwahong, and Saeyeopcil cultivars. Thus, PLS-DA was carried out to enhance the poor separation obtained with the PCA model (Figure 3). PLS-DA is a projection method that separates groups of observations by rotating the PCAs such that a maximum separation among classes, here perilla cultivars, is obtained. Although PLS-DA could not distinguish between Daesil and Daeyeup cultivars, the first and second components resolved the measured composition profiles of Baekkwang, Hwahong, Ipdlkkae1, Okdong, and Saeyeopcil cultivars. In the first component of the PLS-DA, the variation was mainly attributed to β-sitosterol, linolenic acid, and palmitic acid, of which the corresponding loading was positive for all policosanols except hexacosanol and octacosanol and negative for all fatty acids, α-tocopherol, γ-tocopherol, and phytosterols. This suggests that the reasonable score range of the components could be used for sample selection according to the correlation between the variables and these two components.

To determine detailed relationships between the contents of the 24 lipophilic compounds in perilla, we performed Pearson's correlation analyses on the accessions (Figure 4). Significant positive correlations were observed among most of the policosanls or fatty acids. Among the policosanols, octacosanol contents were positively correlated with hexacosanol (r = 0.7842, P < 0.0001) or heptacosanol contents (r = 0.8234, P < 0.0001). The results provided correlations between metabolites that participate in closely related pathways and demonstrated the

robustness of the present experimental system. However, octacosanol and the other lipophilic compounds were not closely associated. Likewise, the linolenic acid contents were positively correlated with α-tocopherol (r = 0.6695, P < 0.001), γ-tocopherol (r = 0.7201, P < 0.001), β-amyrin (r = 0.6410, P < 0.01), and β-sitosterol (r = 0.8367, P < 0.0001) among all perilla accessions. This was consistent with a previous study.[1,21] Kamal-Eldin and Andersson reported a positive correlation between linolenic acid and γ-tocopherol in vegetable oils.[1] Also, a previous study showed that regression using a multiple linear model indicated a positive relationship between polyunsaturated fat and total phytosterols in diet samples.[21] In component 1 of the PCA and PLS-DA, correlation was also found between these compounds through this study, indicating that PCA and PLS-DA could be an excellent tool for easy visualization of complex data.

In conclusion, this study demonstrates the diversity of lipophilic compounds in perilla seeds and identifies correlations between their contents. PCA and PLS-DA were performed to identify differences between the perilla cultivars. Metabolic loading in components 1 and 2, and Pearson's correlation analysis, suggested correlations between the phytochemicals involved in common or closely related pathways. Furthermore, perilla cultivars that had high tocopherol and linolenic acid levels also contained high phytosterol levels excluding policosanol. The potential of metabolite profiling combined with chemometrics as a powerful tool for assessing food quality is demonstrated through this study.

Consumers are aware of the need for a constant supply of phytochemical-containing foods for antioxidant support and disease prevention. This study provides valuable information regarding future conventional and/or genetic breeding programs for perilla containing tocopherol, linolenic acid, and phytosterols. Daesil and Daeyeup cultivars containing relatively high levels of tocopherol, linolenic acid, and phytosterols should be of high dietary value.

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Notes

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