Sa *et al. Applied Biological Chemistry* (2023) 66:41
https://doi.org/10.1186/s13765-023-00801-6

KSABC
The Korean Society for Applied Biological Chemistry

ARTICLE
Open Access

Characterization of volatile compounds of *Perilla* crop (*Perilla frutescens* L.) in South Korea

Kyu Jin Sa[1†], So Jung Jang[1,2†], Sookyeong Lee[3†], Hyun Park[1,2], Jungeun Cho[1,2], Jungsook Sung[3] and Ju Kyong Lee[1,2*]

Abstract

This study was performed to identify and profile the volatile compounds present in three different types of *Perilla* leaves collected from South Korea. Volatile compounds were analyzed by gas-chromatograph-mass spectrometry. In total, 41 volatile compounds were identified belonging to nine chemical classes (six alcohols, seven aldehydes, two benzodioxoles, two esters, three ethers, four ketones, five monoterpenes, one phenylpropanoid, and eleven sesquiterpenes). In cultivated type of var. *frutescens* (CF), weedy type of var. *frutescens* (WF), and weedy type of var. *crispa* (WC), a total of 34, 39, and 41 volatile compounds, respectively, were identified. The predominant compound in CF and WF was perilla ketone (PK; 87.2% and 64.5%, respectively) and in WC was perilla aldehyde (PA; 26.4%). There were 29 and 27 volatile compounds that showed significant differences of content between WC and CF or WF, respectively. In terms of chemotype based on the volatile compounds, CF and WC were PK type and PA or phenylpropanoid (PP) types, respectively. WF accessions, which were PK and PP types in chemical composition, showed intermediate characteristics in the composition of volatile compounds compared with CF and WC. The results obtained in this study identified successfully the composition and content of volatile compounds in *Perilla* crop in South Korea. These results will provide useful information for industries and research related to *Perilla* crop.

Keywords *Perilla frutescens*, *Perilla* leaves, Volatile compounds, Perilla ketone and aldehyde, Gas chromatography–mass spectrometry, South Korea

Introduction

Perilla crop (*Perilla frutescens* L.) is an annual self-fertilizing plant of the Lamiaceae family, and it is divided into two cultivated types based on their morphology and uses in East Asia: *Perilla frutescens* var. *frutescens* used as an oil or vegetable crop and *Perilla frutescens* var. *crispa* used as herbal medicine or a vegetable crop [1–6]. In their morphological characteristics, the cultivated type of var. *frutescens* (CF) has mostly large and soft seeds, green leaves and stems, and non-wrinkly leaves. In contrast, the cultivated type of var. *crispa* (CC) has small and hard seeds and purple or green coloration on the leaves and stems. These two cultivated types (CF, CC) of *Perilla* crop are extensively cultivated and used in South Korea, China, and Japan [3–5, 7]. In South Korea, CF is used as a leafy vegetable crop and for its seed oil while CC is not cultivated because of its declining use as a herbal medicine. However, it is occasionally found as a relict form in South Korea [3, 4]. Moreover, weedy types of *Perilla*

[†]Kyu Jin Sa, So Jung Jang and Sookyeong Lee contributed equally to this work.

*Correspondence:
Ju Kyong Lee
jukyonglee@kangwon.ac.kr
[1] Department of Bio-Resource Sciences, College of Agriculture and Life Sciences, Kangwon National University, Chuncheon 24341, South Korea
[2] Interdisciplinary Program in Smart Agriculture, Kangwon National University, Chuncheon 24341, South Korea
[3] National Agrobiodiversity Center, National Institute of Agricultural Sciences, RDA, 54874 Jeonju, South Korea

crop are commonly found in South Korea in places such as wasteland, roadsides, and around farmers' houses or fields [3, 8]. Although the two cultivated types of *Perilla* crop (TCTPC) can be well distinguished by morphological characteristics such as leaf color and seed size, it is difficult to distinguish between cultivated and weedy types of *Perilla* crop based on morphological traits because they have the same number of chromosomes (2n = 40) and there are intermediate types resulting from natural hybridization [9–12].

Essential oils are naturally occurring volatile compounds with unique odors and are a complex mixture of secondary metabolites that are synthesized and produced by aromatic and spice plants [13, 14]. Volatile compounds, together with sugars and acids, are the main chemical compounds that determine the characteristic aroma and flavor of foods [15]. The essential oil of *Perilla* leaves contains around 150–200 different compounds, which have high antioxidant, anticancer, anti-inflammatory, insecticidal, and antimicrobial activities [16]. The pharmacological activities of *Perilla* crop are caused by the content and composition of volatile compounds, which are determined by the chemotype, geographical location, growing environment, growth stage, collection zone, and extraction method [17, 18]. *Perilla* leaves are an important source of volatile compounds and a rich source of perilla aldehyde (PA), perilla ketone (PK), β-caryophyllene, benzaldehyde, and limonene [19] Depending on the varying content of these compounds, *Perilla* plants are categorized into seven chemotypes based on the volatile compounds present in their leaves: PA, PK (isoegomaketone), elsholtzia ketone (EK), citral (C), perillene (PL), piperitenone (PT), and phenylpropanoid (PP; myristicin, dill apiole, elemicin) [20–22]. Among these compounds, PA is the most abundant compound in CC, and it is used as a food additive for flavor and spiciness and in various medicinal applications for its potent antimicrobial properties against both *Candida albicans* and airborne microbes [18, 23–25]. Moreover, PK is also a major compound for flavor activity in CF, which has potent acute toxicity and cardio-pulmonary toxicity for animals and livestock [26]. Myristicin is a major compound of the CC that is collected in South Korea [27]. It is widely accepted that elemicin and myristicin are psychoactive compounds [16]. In addition, elemicin and dill apiole have been found to be effective in inhibiting proinflammatory cytokines during lung inflammation [16]. Myristicin and dill-apiol are most potent in hexobarbital induced sleeping time prolongation observed in mice [28]. A previous study identified 1-octen-3-ol compound as a key compound in Korean perilla leaves [29]. Another compound, (Z,E)-a-farnesene, has been identified as a major compound in both Chinese CF and CC [18].

Differences in volatile compounds can result in varying physiological effects, and these differences can impact the utilization of the compounds in food and pharmaceutical products [30]. To develop new *Perilla* varieties with high levels of useful volatile compounds for health, it is essential to conduct research on the content and composition of the volatile compounds in breeding materials. Therefore, we report on the variability of volatile compounds in three different types [CF, weedy type of var. *frutescens* (WF), weedy type of var. *crispa* (WC)] of *Perilla* crop collected from South Korea.

Materials and methods

Plant materials for volatile profile analysis

Information on the number of accessions and their locations for a total of 80 *Perilla* samples collected in northern, central, and southern regions of South Korea are shown in Table 1 and Addition file 1: Fig. S1. These accessions comprised 40 samples of CF, 20 samples of WF, and 20 samples of WC. This study tentatively categorized the *Perilla* samples as either the cultivated or weedy type based on a previous study [3]. All of the CF accessions were landraces collected from farms in the various regions in South Korea. In addition, WF and WC were collected on roadsides, in waste areas, and around farmers' fields or farmhouses in South Korea. In 2022, we cultivated 80 accessions of *Perilla* crop at the experimental farm of Kangwon National University in Chuncheon, Gangwon-do, and collected leaf samples of each accession during the flowering period.

Profiling of volatile compounds using gas chromatography-mass spectrometry (*GC–MS)*

Volatile compounds were analyzed using a headspace solid-phase microextraction (HS-SPME) injector-equipped Trace 1310 gas chromatograph and TSQ 8000 triple quadrupole mass spectrometer (Thermo-scientific, San Jose, CA, USA) and TriPlus RSH autosampler (Waltham, MA, USA) with a DB-Wax capillary column (60 m × 0.25 mm, 0.50 µm; Agilent Technologies). For analysis of the volatile compounds, leaves of CF, WF, and WC were harvested and ground using a Freezer/Mill 6875 (SPEX SamplePrep, Metuchen, NJ, USA). The ground leaves (0.5 g) for each sample were incubated for 10 min at 60 °C. Headspace volatiles of the *Perilla* leaf samples were adsorbed by inserting SPME fiber assemblies (Supelco, PDMS/DVB; Sigma–Aldrich, St. Louis, MO, USA) into the vial for 10 min at 60 °C with agitation. The injector temperature was 250 °C. The temperature of the gas chromatograph oven was initially maintained at 40 °C for 2 min, ramped to 150 °C at 4 °C/min, held for 10 min, then ramped to 200 °C at 4 °C/min, held for 5 min,

Table 1 *Perilla* accessions from different area of South Korea used for volatile compounds profiling

Code no.	Accession no.	City and province	Type
1	PF20-057	Dangjin-si, Chungcheongnam-do	Cultivated type of var. *frutescens*
2	PF20-058	Dangjin-si, Chungcheongnam-do	Cultivated type of var. *frutescens*
3	PF20-061	Seosan-si, Chungcheongnam-do	Cultivated type of var. *frutescens*
4	PF20-062	Seosan-si, Chungcheongnam-do	Cultivated type of var. *frutescens*
5	PF20-065	Seosan-si, Chungcheongnam-do	Cultivated type of var. *frutescens*
6	PF20-075	Hongseong-gun, Chungcheongnam-do	Cultivated type of var. *frutescens*
7	PF20-076	Yesan-gun, Chungcheongnam-do	Cultivated type of var. *frutescens*
8	PF20-077	Yesan-gun, Chungcheongnam-do	Cultivated type of var. *frutescens*
9	PF20-078	Yesan-gun, Chungcheongnam-do	Cultivated type of var. *frutescens*
10	PF20-081	Cheongyang-gun, Chungcheongnam-do	Cultivated type of var. *frutescens*
11	PF20-086	Cheongyang-gun, Chungcheongnam-do	Cultivated type of var. *frutescens*
12	PF20-088	Hongseong-gun, Chungcheongnam-do	Cultivated type of var. *frutescens*
13	PF20-090	Cheongyang-gun, Chungcheongnam-do	Cultivated type of var. *frutescens*
14	PF20-096	Boryeong-si, Chungcheongnam-do	Cultivated type of var. *frutescens*
15	PF20-097	Boryeong-si, Chungcheongnam-do	Cultivated type of var. *frutescens*
16	PF20-100	Boryeong-si, Chungcheongnam-do	Cultivated type of var. *frutescens*
17	PF20-102	Seocheon-gun, Chungcheongnam-do	Cultivated type of var. *frutescens*
18	PF20-104	Seocheon-gun, Chungcheongnam-do	Cultivated type of var. *frutescens*
19	PF20-109	Seocheon-gun, Chungcheongnam-do	Cultivated type of var. *frutescens*
20	PF20-113	Gunsan-si, Jeollabuk-do	Cultivated type of var. *frutescens*
21	PF20-116	Gunsan-si, Jeollabuk-do	Cultivated type of var. *frutescens*
22	PF20-118	Gunsan-si, Jeollabuk-do	Cultivated type of var. *frutescens*
23	PF20-120	Gunsan-si, Jeollabuk-do	Cultivated type of var. *frutescens*
24	PF20-123	Gunsan-si, Jeollabuk-do	Cultivated type of var. *frutescens*
25	PF20-125	Gunsan-si, Jeollabuk-do	Cultivated type of var. *frutescens*
26	PF20-127	Gunsan-si, Jeollabuk-do	Cultivated type of var. *frutescens*
27	PF20-143	Nonsan-si, Chungcheongnam-do	Cultivated type of var. *frutescens*
28	PF20-145	Nonsan-si, Chungcheongnam-do	Cultivated type of var. *frutescens*
29	PF20-151	Nonsan-si, Chungcheongnam-do	Cultivated type of var. *frutescens*
30	PF21-001	Sancheong-gun, Gyeongsangnam-do	Cultivated type of var. *frutescens*
31	PF21-002	Sancheong-gun, Gyeongsangnam-do	Cultivated type of var. *frutescens*
32	PF21-004	Sancheong-gun, Gyeongsangnam-do	Cultivated type of var. *frutescens*
33	PF21-008	Sancheong-gun, Gyeongsangnam-do	Cultivated type of var. *frutescens*
34	PF21-013	Hadong-gun, Gyeongsangnam-do	Cultivated type of var. *frutescens*
35	PF21-015	Hadong-gun, Gyeongsangnam-do	Cultivated type of var. *frutescens*
36	PF21-020	Gurye-gun, Jeollanam-do	Cultivated type of var. *frutescens*
37	PF21-022	Gurye-gun, Jeollanam-do	Cultivated type of var. *frutescens*
38	PF21-025	Gurye-gun, Jeollanam-do	Cultivated type of var. *frutescens*
39	PF21-026	Suncheon-si, Jeollanam-do	Cultivated type of var. *frutescens*
40	PF21-027	Suncheon-si, Jeollanam-do	Cultivated type of var. *frutescens*
41	PF20-005	Yangpyeong-gun, Gyeonggi-do	Weedy type of var. *frutescens*
42	PF20-024	Yeoju-si, Gyeonggi-do	Weedy type of var. *frutescens*
43	PF20-026	Yeoju-si, Gyeonggi-do	Weedy type of var. *frutescens*
44	PF20-039	Icheon-si, Gyeonggi-do	Weedy type of var. *frutescens*
45	PF20-083	Cheongyang-gun, Chungcheongnam-do	Weedy type of var. *frutescens*
46	PF20-085	Cheongyang-gun, Chungcheongnam-do	Weedy type of var. *frutescens*
47	PF20-095	Boryeong-si, Chungcheongnam-do	Weedy type of var. *frutescens*
48	PF20-098	Boryeong-si, Chungcheongnam-do	Weedy type of var. *frutescens*
49	PF20-103	Seocheon-gun, Chungcheongnam-do	Weedy type of var. *frutescens*

Table 1 (continued)

Code no.	Accession no.	City and province	Type
50	PF20-106	Seocheon-gun, Chungcheongnam-do	Weedy type of var. *frutescens*
51	PF20-112	Seocheon-gun, Chungcheongnam-do	Weedy type of var. *frutescens*
52	PF20-115	Gunsan-si, Jeollabuk-do	Weedy type of var. *frutescens*
53	PF20-121	Gunsan-si, Jeollabuk-do	Weedy type of var. *frutescens*
54	PF20-137	Nonsan-si, Chungcheongnam-do	Weedy type of var. *frutescens*
55	PF20-142	Nonsan-si, Chungcheongnam-do	Weedy type of var. *frutescens*
56	PF20-149	Nonsan-si, Chungcheongnam-do	Weedy type of var. *frutescens*
57	PF20-153	Nonsan-si, Chungcheongnam-do	Weedy type of var. *frutescens*
58	PF21-010	Hadong-gun, Gyeongsangnam-do	Weedy type of var. *frutescens*
59	PF19-133	Hapcheon-gun, Gyeongsangnam-do	Weedy type of var. *frutescens*
60	PF20-011	Yangpyeong-gun, Gyeonggi-do	Weedy type of var. *frutescens*
61	PF20-082	Cheongyang-gun, Chungcheongnam-do	Weedy type of var. *crispa*
62	PF20-091	Boryeong-si, Chungcheongnam-do	Weedy type of var. *crispa*
63	PF20-094	Boryeong-si, Chungcheongnam-do	Weedy type of var. *crispa*
64	PF20-099	Boryeong-si, Chungcheongnam-do	Weedy type of var. *crispa*
65	PF20-101	Seocheon-gun, Chungcheongnam-do	Weedy type of var. *crispa*
66	PF20-128	Gunsan-si, Jeollabuk-do	Weedy type of var. *crispa*
67	PF20-141	Nonsan-si, Chungcheongnam-do	Weedy type of var. *crispa*
68	PF20-150	Nonsan-si, Chungcheongnam-do	Weedy type of var. *crispa*
69	PF20-152	Nonsan-si, Chungcheongnam-do	Weedy type of var. *crispa*
70	PF21-005	Sancheong-gun, Gyeongsangnam-do	Weedy type of var. *crispa*
71	PF21-012	Hadong-gun, Gyeongsangnam-do	Weedy type of var. *crispa*
72	PF21-017	Hadong-gun, Gyeongsangnam-do	Weedy type of var. *crispa*
73	PF21-018	Gurye-gun, Jeollanam-do	Weedy type of var. *crispa*
74	PF21-023	Gurye-gun, Jeollanam-do	Weedy type of var. *crispa*
75	PF21-028	Suncheon-si, Jeollanam-do	Weedy type of var. *crispa*
76	PF19-114	Changnyeong-gun, Gyeongsangnam-do	Weedy type of var. *crispa*
77	PF16-123	Muju-gun, Jeollabuk-do	Weedy type of var. *crispa*
78	PF17-039	Sunchang-gun, Jeollabuk-do	Weedy type of var. *crispa*
79	PF18-007	Geumsan-gun, Chungcheongnam-do	Weedy type of var. *crispa*
80	PF18-011	Geumsan-gun, Chungcheongnam-do	Weedy type of var. *crispa*

and finally ramped to 230 °C at 10 °C/min and held for 5 min. Total run time was 65 min, and the helium carrier gas flow was maintained constant at 1.5 mL/min in the splitless with purge mode during the analysis. Total 41 volatile compounds from the leaf samples were identified based on the MS database (NIST version 2.0) with a similarity index (SI) higher than 70%. Similarity index less than 70% was not considered. Quantitative analysis of the percentage of each volatile compound was calculated using the formula below:

$$\text{Relative content} = M/N * 100\%$$

where M is the peak area of the individual volatile compounds and N is the identified total peak area.

Statistical analysis

The relative content of the identified compounds was employed for analysis. Analysis of variance (ANOVA), correlation analysis, principal component analysis (PCA), hierarchical cluster analysis (HCA), and K-means clustering of gas chromatography–mass spectrometry (GC–MS) data were conducted using MetaboAnalyst 5.0, a web-based platform [31]. The significance of differences in the mean values for the various *Perilla* types was evaluated using Fisher's LSD test ($p < 0.05$). To calculate the loading values of eigenvectors and identify the major statistically different components among the samples, PCA was employed. The goal of K-means clustering is to generate k clusters in such a way that the total squared distance between each point and its assigned cluster center is minimized. A dendrogram was generated using Ward's method of hierarchical clustering and Euclidean distance measurement between the analyzed samples, through HCA analysis of the GC-MS data.

Results

GC–MS Profiling of Volatile Compounds of *Perilla* leaf

Volatile profiles of 80 *Perilla* accessions collected in South Korea were analyzed by GC–MS and 41 volatile compounds were identified. These volatile compounds belonged to nine chemical classes: six alcohols, seven aldehydes, two benzodioxoles, two esters, three ethers, four ketones, five monoterpenes, one phenylpropanoid, and eleven sesquiterpenes (Table 2, Additional file 2: Tables S1 and S2, Fig. 1). Ketones comprised 61.7% of the identified compounds, with a predominance of PK in all *Perilla* accessions. Aldehydes represented approximately 9.7% of the total identified compounds, with the dominance of PA (Table 2; Fig. 1).

This study also investigated the characteristics of the volatile compounds of the three subgroups of *Perilla*. The highest number of compounds (41) was identified in WC. The aldehydes and benzodioxoles were the most prominent, representing 32.5% and 20.0%, respectively, in WC. A total of 39 compounds were identified in WF with 68.1% being ketones and 12.3% being benzodioxoles. The lowest number of compounds was detected in CF with 34 compounds, which included a high proportion of ketones (87.5%) (Table 2, Additional file 2: Table S2, Fig. 1). Among these compounds, the predominant compound of CF was PK (87.2%). The top three compounds of WF were PK (64.5%), myristicin (7.0%), and dill apiol (5.3%). The main components of WC were PA (26.4%), dill apiol (13.8%), (Z,E)-α-farnesene (9.2%), 1-octen-3-ol (6.2%), and myristicin (5.7%) (Table 2, Additional file 2: Table S1).

Significant differences and correlation analysis of volatile compounds in *Perilla* crop

This study confirmed significant differences among the three different types (CF, WF, WC) of *Perilla* accessions for the 41 volatile compounds (Table 2). No significant differences were found among the three types of *Perilla* accessions for myristicin, methyl salicylate, 3-(4-methyl-3-pentenyl)furan, elemicin, and (E)-β-farnesene. Between WF and CF, five compounds (1-penten-3-ol, 2-penten-1-ol, hexanal, dill apiol, and linalool) exhibited significant differences, while between WF and CF isoegomaketone showed a significant difference. Moreover, between WC and WF and between WC and CF 24 compounds (1-hexanol, (Z)-hex-3-en-1-ol, 1-octen-3-ol, shisool, 2-methylbutanal, β-methylbutanal, E-2-pentenal, (E)-2-hexenal, 2,4-hexadienal, PA, methyl benzoate, 2-ethylfuran, 1-penten-3-one, 6-methyl-5-heptene-2-one, β-pinene, β-myrcene, D-limonene, α-terpineol, eugenol, γ-elemene, bicyclogermacrene, caryophyllene oxide, (Z,E)-α-farnesene, (E)-γ-bisabolene) showed significant differences. One compound, copaene, exhibited significant differences between WC and CF, as well as between WF and CF. Another compound, β-bourbonene, showed significant differences between WF and CF and between WC and CF. The compounds caryophyllene, α-humulene, nerolidol, and PK also showed significant differences among the three types of *Perilla* accessions (Table 2).

This study evaluated correlation coefficients among the 41 volatile compounds in the 80 *Perilla* accessions (Fig. 2). Correlation analysis was performed to confirm

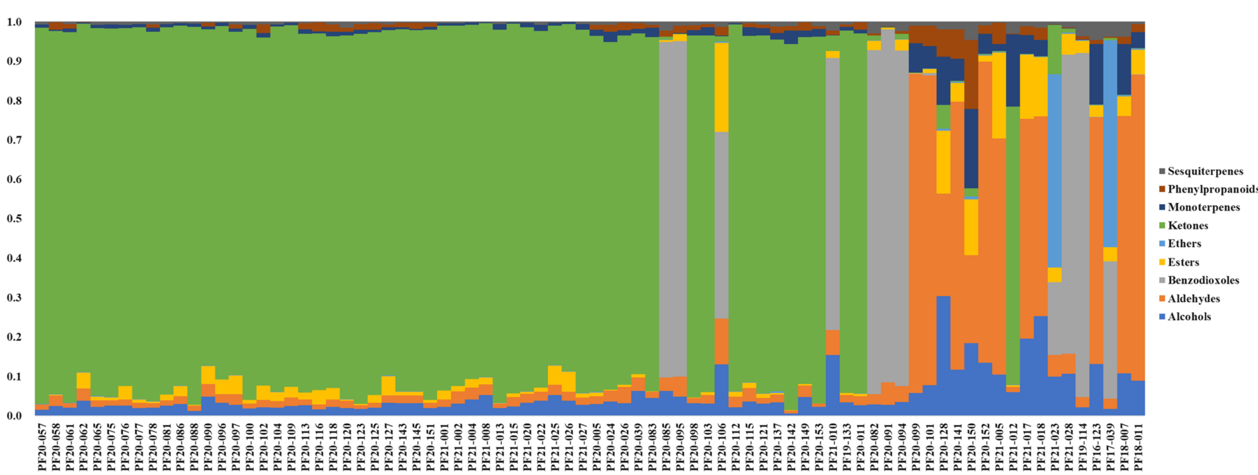

Fig. 1 Bar plot of ratio of each chemical class for the identified volatile compounds in *Perilla* accessions. CF: cultivated type of var. *frutescens*; WF: weedy type of var. *frutescens*; WC: weedy type of var. *crispa*

Table 2 Composition and comparison of volatile compounds of three types of *Perilla* accessions

Volatile Compound	Retention time	Chemical class	All accessions (n = 80)	CF (n = 40)	WF (n = 20)	WC (n = 20)	Statistical comparison[a]
1-Penten-3-ol	17.16	Alcohols	0.219 ± 0.297	0.132 ± 0.085	0.220 ± 0.381	0.391 ± 0.399	WC ≠ CF
2-Penten-1-ol	23.12	Alcohols	0.124 ± 0.160	0.079 ± 0.058	0.122 ± 0.200	0.216 ± 0.216	WC ≠ CF
1-Hexanol	24.22	Alcohols	0.019 ± 0.019	0.013 ± 0.007	0.017 ± 0.018	0.032 ± 0.028	WC ≠ CF; WC ≠ WF
(Z)-Hex-3-en-1-ol	25.45	Alcohols	0.238 ± 0.258	0.168 ± 0.090	0.188 ± 0.186	0.427 ± 0.418	WC ≠ CF; WC ≠ WF
1-Octen-3-ol	27.57	Alcohols	3.594 ± 3.581	2.261 ± 0.812	3.650 ± 2.362	6.203 ± 5.956	WC ≠ CF; WC ≠ WF
Shisool	48.65	Alcohols	0.457 ± 1.318	-	0.008 ± 0.034	1.820 ± 2.149	WC ≠ CF; WC ≠ WF
2-Methylbutanal	8.77	Aldehydes	0.008 ± 0.008	0.005 ± 0.003	0.008 ± 0.007	0.013 ± 0.013	WC ≠ CF; WC ≠ WF
β-Methylbutanal	8.89	Aldehydes	0.015 ± 0.018	0.010 ± 0.005	0.013 ± 0.012	0.027 ± 0.032	WC ≠ CF; WC ≠ WF
Hexanal	14.55	Aldehydes	0.195 ± 0.272	0.127 ± 0.081	0.199 ± 0.321	0.328 ± 0.403	WC ≠ CF
E-2-pentenal	16.45	Aldehydes	0.018 ± 0.022	0.013 ± 0.007	0.015 ± 0.018	0.033 ± 0.035	WC ≠ CF; WC ≠ WF
(E)-2-Hexenal	19.85	Aldehydes	0.805 ± 0.905	0.458 ± 0.232	0.699 ± 0.624	1.607 ± 1.402	WC ≠ CF; WC ≠ WF
2,4-Hexadienal	26.63	Aldehydes	2.072 ± 2.124	1.220 ± 0.387	1.787 ± 1.165	4.061 ± 3.365	WC ≠ CF; WC ≠ WF
Perilla aldehyde	43.96	Aldehydes	6.635 ± 17.988	-	0.117 ± 0.524	26.422 ± 28.205	WC ≠ CF; WC ≠ WF
Myristicin	61.63	Benzodioxoles	3.165 ± 12.428	-	6.974 ± 19.683	5.688 ± 14.550	ns.
Dill apiol	64.44	Benzodioxoles	4.786 ± 15.849	-	5.311 ± 13.548	13.832 ± 26.948	WC ≠ CF
Methyl benzoate	34.91	Esters	1.063 ± 3.179	0.023 ± 0.020	0.433 ± 1.434	3.773 ± 5.435	WC ≠ CF; WC ≠ WF
Methyl salicylate	43.49	Esters	1.607 ± 2.139	1.825 ± 1.530	1.160 ± 2.954	1.618 ± 2.275	ns.
2-Ethylfuran	10.02	Ethers	0.069 ± 0.072	0.042 ± 0.019	0.059 ± 0.067	0.132 ± 0.103	WC ≠ CF; WC ≠ WF
3-(4-Methyl-3-pentenyl)furan	26.89	Ethers	0.019 ± 0.044	0.018 ± 0.029	0.029 ± 0.073	0.012 ± 0.029	ns.
Elemicin	60.32	Ethers	1.059 ± 6.656	-	-	4.235 ± 13.040	ns.
1-Penten-3-one	12.36	Ketones	0.042 ± 0.048	0.032 ± 0.023	0.035 ± 0.037	0.071 ± 0.077	WC ≠ CF; WC ≠ WF
6-Methyl-5-heptene-2-one	24.03	Ketones	0.032 ± 0.057	0.014 ± 0.008	0.024 ± 0.028	0.074 ± 0.100	WC ≠ CF; WC ≠ WF
Perilla ketone	43.87	Ketones	60.462 ± 38.721	87.232 ± 2.894	64.482 ± 33.582	2.902 ± 8.714	CF ≠ WC; CF ≠ WF; WF ≠ WC
Isoegomaketone	49.77	Ketones	1.194 ± 4.913	0.185 ± 0.135	3.572 ± 8.889	0.832 ± 3.618	WF ≠ CF
β-Pinene	15.47	Monoterpenes	0.028 ± 0.093	-	0.000 ± 0.001	0.111 ± 0.162	WC ≠ CF; WC ≠ WF
β-Myrcene	17.43	Monoterpenes	0.035 ± 0.064	0.013 ± 0.009	0.027 ± 0.018	0.086 ± 0.112	WC ≠ CF; WC ≠ WF
D-Limonene	18.95	Monoterpenes	0.517 ± 1.604	0.004 ± 0.001	0.011 ± 0.020	2.051 ± 2.719	WC ≠ CF; WC ≠ WF
Linalool	30.86	Monoterpenes	1.231 ± 1.855	0.742 ± 0.278	1.265 ± 0.845	2.175 ± 3.463	WC ≠ CF
α-Terpineol	38.15	Monoterpenes	0.058 ± 0.154	-	-	0.233 ± 0.236	WC ≠ CF; WC ≠ WF
Eugenol	58.78	Phenylpropanoids	1.200 ± 1.792	0.531 ± 0.719	1.190 ± 0.528	2.548 ± 3.027	WC ≠ CF; WC ≠ WF
γ-Elemene	29.06	Sesquiterpenes	0.020 ± 0.025	0.013 ± 0.009	0.018 ± 0.022	0.035 ± 0.040	WC ≠ CF; WC ≠ WF
Copaene	29.57	Sesquiterpenes	0.045 ± 0.051	0.029 ± 0.022	0.056 ± 0.065	0.067 ± 0.067	WC ≠ CF; WF ≠ CF
β-Bourbonene	30.54	Sesquiterpenes	0.058 ± 0.078	0.042 ± 0.040	0.103 ± 0.124	0.044 ± 0.060	WF ≠ CF; WF ≠ WC
Caryophyllene	33.75	Sesquiterpenes	2.330 ± 2.303	1.001 ± 0.644	2.451 ± 1.721	4.865 ± 2.783	WC ≠ CF; WF ≠ CF; WC ≠ WF
α-Humulene	37.36	Sesquiterpenes	0.702 ± 0.685	0.323 ± 0.198	0.733 ± 0.511	1.428 ± 0.866	WC ≠ CF; WF ≠ CF; WC ≠ WF
bicyclogermacrene	40.96	Sesquiterpenes	0.229 ± 0.252	0.155 ± 0.104	0.212 ± 0.213	0.395 ± 0.395	WC ≠ CF; WC ≠ WF
Caryophyllene oxide	52.05	Sesquiterpenes	0.099 ± 0.201	0.028 ± 0.008	0.078 ± 0.090	0.263 ± 0.347	WC ≠ CF; WC ≠ WF
Nerolidol	53.27	Sesquiterpenes	0.142 ± 0.124	0.077 ± 0.031	0.141 ± 0.100	0.275 ± 0.157	WC ≠ CF; WF ≠ CF; WC ≠ WF
(E)-β-Farnesene	36.13	Sesquiterpenes	0.381 ± 1.578	0.013 ± 0.010	0.638 ± 2.439	0.863 ± 1.951	ns.
(Z,E)-α-Farnesene	39.48	Sesquiterpenes	4.711 ± 4.243	2.981 ± 1.788	3.707 ± 2.947	9.176 ± 5.591	WC ≠ CF; WC ≠ WF
(E)-γ-Bisabolene	47.98	Sesquiterpenes	0.317 ± 0.310	0.192 ± 0.124	0.250 ± 0.225	0.635 ± 0.417	WC ≠ CF; WC ≠ WF

CF: Cultivated type of var. *frutescenes*; WF: Weedy type of var. *frutescens*; WC: Weedy type of var. *crispa*

[a] Statistical significance among three different types of *Perilla* accessions. Comparison was performed with ANOVA followed by Fisher's LDS ($p < 0.05$)

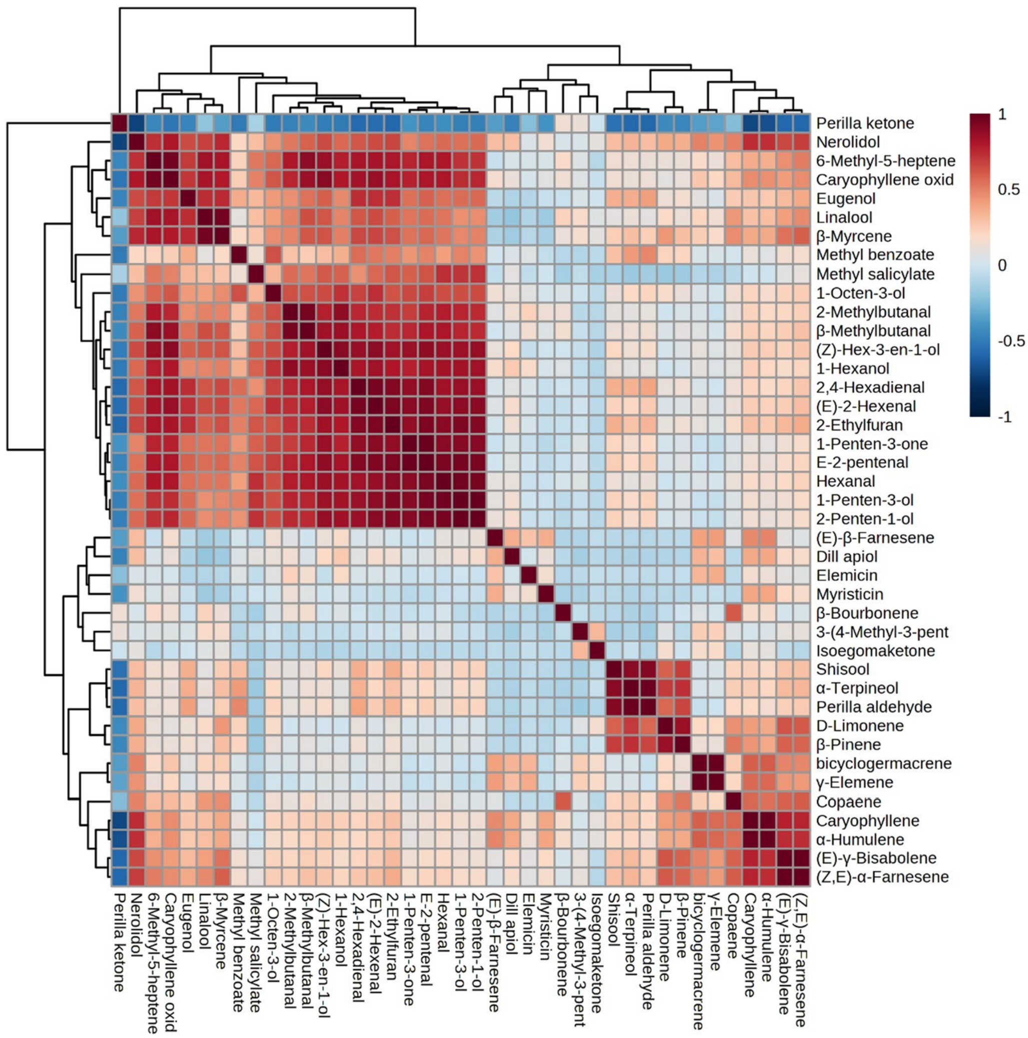

Fig. 2 Correlation heatmap of the 41 volatile compounds in80 *Perilla* accessions

genetic relationships among the various volatile compounds. Among all 820 combinations, 78 combinations showed comparatively higher positive correlation coefficients (over 0.800 at $p<0.01$) than the other combinations. Moreover, two combinations showed relatively higher negative correlation coefficients (less 0.7), between PK and nerolidol (− 0.725 at $p<0.01$) and between PK and caryophyllene (− 0.711 at $p<0.01$).

PCA and HCA of GC–MS profiling data

To confirm the differences or relationships among the *Perilla* accessions and to examine which identified volatile compounds were responsible for the major contribution to the difference in *Perilla frutescens* var. *frutescens* and var. *crispa*, PCA and HCA of the detected compounds were performed. The PCA results demonstrated that the first and second principal components accounted for 86.3% of the variance [PC1 (72.5%), PC2 (13.8%)] of all identified volatile compounds in the *Perilla* accessions (Fig. 3A). PK exhibited highly negative loading scores (− 0.933) on PC1, which enabled the differentiation of all CF and almost all WF accessions from the remaining ones. Volatile compounds with highly positive or negative scores on PC2 were PA (0.731), dill apiol (− 0.623), and myristicin (− 0.245), which enabled all WC and a few WF accessions to be distinguished (Fig. 3B, Additional file 2: Table S3). When K-means clustering was performed with K = 3 on a dataset of the 80 *Perilla* accessions (Table 3), three clusters were obtained. Cluster 1 consisted of nine WC and four WF accessions, while Cluster 2 comprised all 40 CF, 16 WF, and one WC accession. Cluster 3 contained only 10 WC accessions.

HCA was performed to understand the relationships between the analyzed *Perilla* accessions. According to the dendrogram and heatmap obtained (Figs. 4 and 5), all *Perilla* accessions could be divided into two main groups

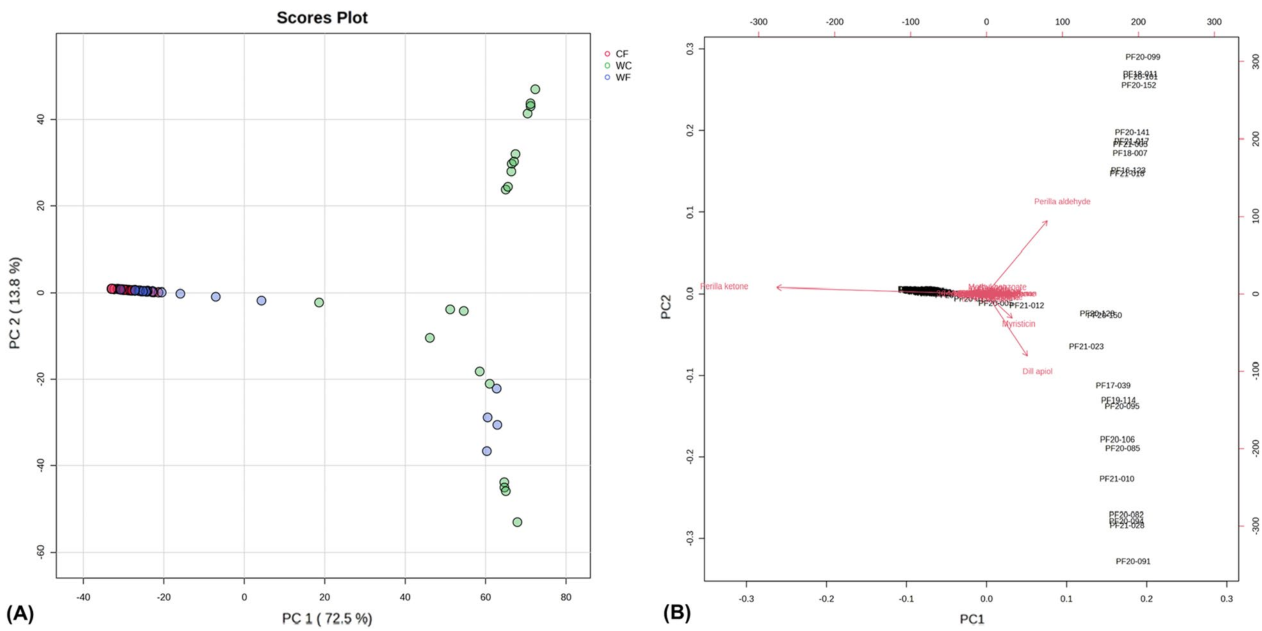

Fig. 3 PCA score plot for volatile compounds among 80 *Perilla* accessions (**A**), and loading plot for volatile compounds explaining the variation on PC1 and PC2 axes (**B**)

Table 3 K-mean clustering in K = 3 of *Perilla* accessions using volatile compounds

Cluster	Members*
Cluster 1	PF20-082, PF20-091, PF20-094, PF20-128, PF20-150, PF21-023, PF21-028, PF19-114, PF17-039, PF20-085, PF20-095, PF20-106, PF21-010
Cluster 2	PF20-057, PF20-058, PF20-061, PF20-062, PF20-065, PF20-075, PF20-076, PF20-077, PF20-078, PF20-081, PF20-086, PF20-088, PF20-090, PF20-096, PF20-097, PF20-100, PF20-102, PF20-104, PF20-109, PF20-113, PF20-116, PF20-118, PF20-120, PF20-123, PF20-125, PF20-127, PF20-143, PF20-145, PF20-151, PF21-001, PF21-002, PF21-004, PF21-008, PF21-013, PF21-015, PF21-020, PF21-022, PF21-025, PF21-026, PF21-027, PF21-012, PF20-005, PF20-024, PF20-026, PF20-039, PF20-083, PF20-098, PF20-103, PF20-112, PF20-115, PF20-121, PF20-137, PF20-142, PF20-149, PF20-153, PF19-133, PF20-011
Cluster 3	PF20-099, PF20-101, PF20-141, PF20-152, PF21-005, PF21-017, PF21-018, PF16-123, PF18-007, PF18-011

*The red, blue, and green letters represent CF, WF, and WC, respectively

(Group I and II), and these correspond to var. *frutescens* and var. *crispa* except for five accessions (PF20-095, PF20-085, PF21-010, PF20-106, PF20-112) (Figs. 4 and 5). In detail, Group I contained 23 accessions with four WF and 19 WC, while Group II included 40 CF, 16 WF, and one WC (Figs. 4 and 5).

Discussion

Growing demand for functional foods among consumers has driven a greater research emphasis on investigating the physiological and pharmacological properties of compounds derived from plants. *Perilla* exhibits significant pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, antidepressant, anxiolytic, chemo-preventive, and antitumor-promoting effects [32–34], The presence of bioactive compounds, such as flavonoids, essential oils (volatile compounds), unsaturated fatty acids, triterpenes, and phenolic compounds in *Perilla* plants is responsible for their pharmacological activities [26]. *Perilla*'s volatile compounds in particular are considered key components that contribute to its medicinal properties, aroma, and flavor [18, 35]. Therefore, they are commonly utilized in the manufacture of pharmaceuticals and food additives [36, 37].

This study identified a total of 41 volatile compounds belonging to nine chemical classes in this population using GC-MS (Table 2, Additional file 2: Table S1). Although all 41 compounds were identified in 20 accessions of WC, seven (shisool, PA, myristicin, dill apiol, elemicin, β-pinene, α-terpineol) compounds were not detected in CF (Table 2). Comparing the statistical differences of commonly identified compounds between CF and WC, 29 volatile compounds showed significant differences in contents between the two types (Table 2). This suggests that different types of *Perilla* may exhibit varying aromas, flavors, and pharmacological effects. Both

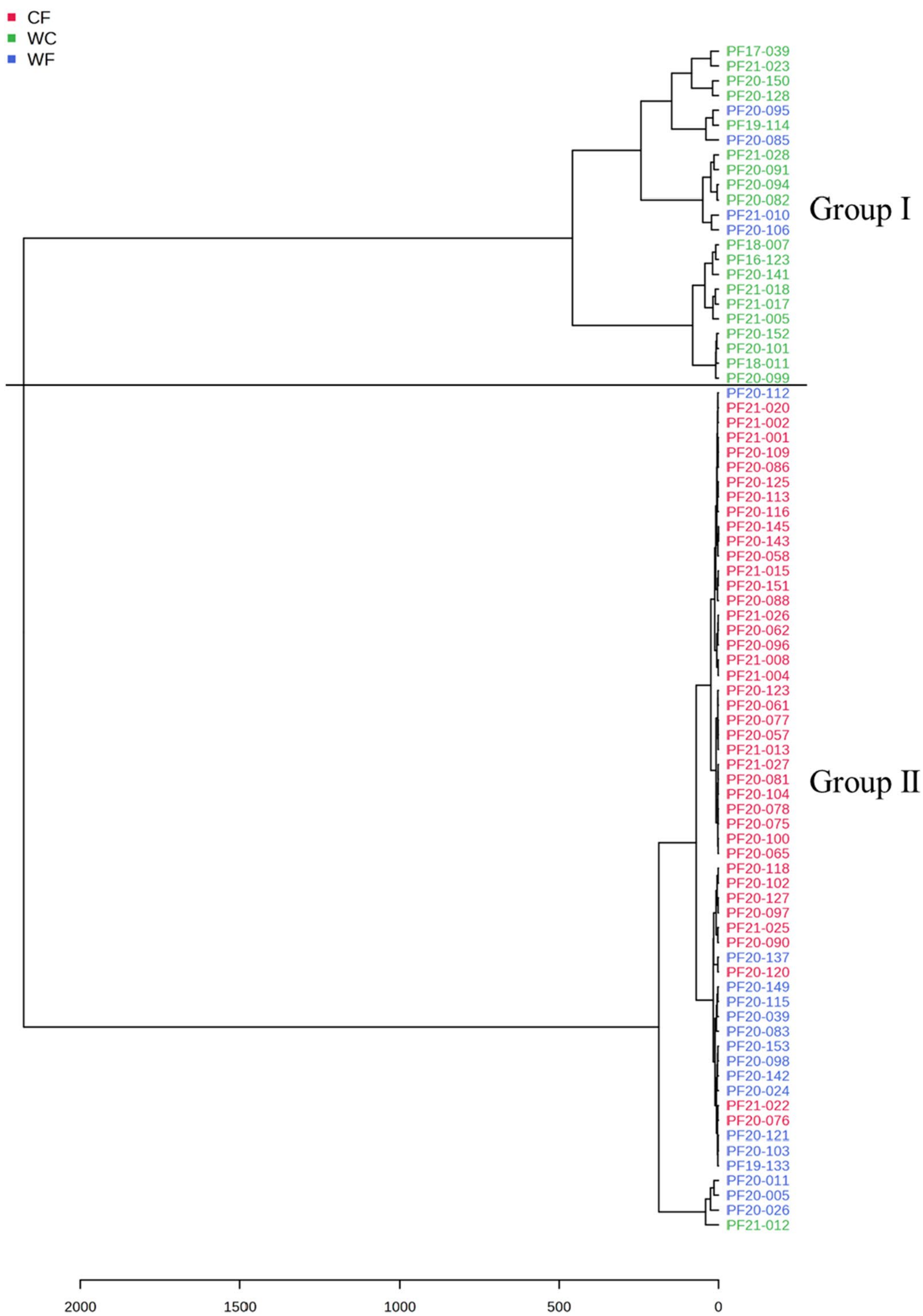

Fig. 4 Dendrogram obtained by hierarchical clusteranalysis on volatile components of 80 *Perilla*accessions

the green-leafed CF and the purple-leafed CC are generally recognized as separate species by botanists [18], and their Korean names are “Deulggae” or “Kkaennip” and “Chajoki” or “Soyup”, respectively. In South Korea, *Perilla* leaves of CF are mainly used as leafy vegetables, while those of CC are used for medicinal purposes. Moreover, the green-leafed Korean variety of *Perilla* was the least bitter with a high intensity of cooling sensation, while

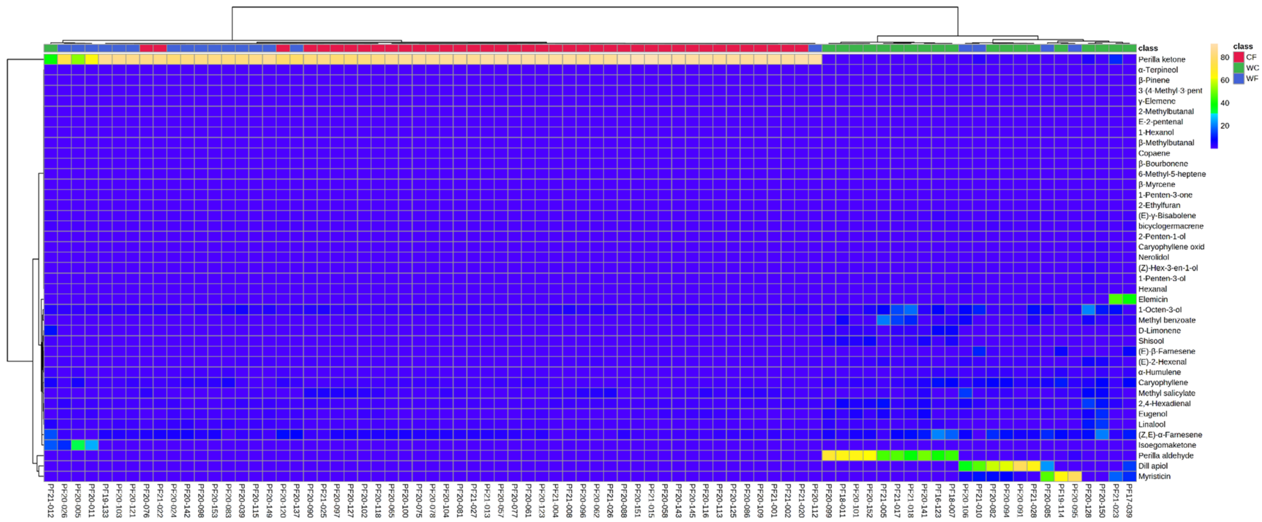

Fig. 5 Heatmap with hierarchical clustering with 41volatile compounds in *Perilla*accessions

the red-leaved *Perilla* was the least astringent and pungent according to a sensory test by electronic nose sensors [38]. The differences in chemical compounds are reflected in the distinct uses and sensory diversity of CF and WC.

Moreover, the predominant compound of CF accessions, with a very high proportion, was PK (80.1%~92.1%). Then, depending on the accession, the next most prominent compounds were (Z,E)-α-farnesene (0.3%~8.8%), methyl salicylate (0.03%~5.0%), and 1-octen-3-ol (1.1%~4.6%) with relatively low proportions (Additional file 2: Table S1). Based on the seven chemotypes for volatile compounds (PA, PK, EK, C, PL, PT, and PP), all of the CF in this study were of the PK type (PK with no isoegomaketone) (Fig. 5, Additional file 2: Table S1). These results may be an indication that the level of diversity for volatile compounds has been reduced by direct or indirect strong selection by people and genetic drift during the domestication from wild or weedy to cultivated type. A previous study confirmed the presence of volatile compounds among five Korean *Perilla* cultivars (cv. Namcheon, Bora, Saebora, Dongle1, and Dongle2) with purple leaves. The predominant compound was PK, along with some isoegomaketone [39]. The CF accessions used in this study were all landraces collected from farmers. These results suggest that Koreans may prefer the aroma of PK over other aroma types, such as PA, for vegetable uses.

The WC accessions in this study had more variation in volatile compounds compared with the CF accessions, with a combination of various compounds being identified in WC (Fig. 1, Additional file 2: Tables S1 and S2). Although most accessions contained PA and PP types, some accessions were clearly divided into different chemotypes. For example, nine accessions (PF20-099, PF18-011, PF20-101, and PF20-152 for PA type; PF20-091, PF21-028, PF20-094, and PF20-082 for PP type of dill apiol; PF19-114 for PP type of myristicin) have a high relative content of PA (62.5%~70.3%), dill apiol (58.5%~77.3), and myristicin (63.9%) with a ratio of over 50%. Although eight WC accessions (PF20-141, PF21-017, PF21-005, PF18-007, PF16-123, and PF21-018 for PA type; PF17-039 and PF21-023 for PP type of elemicin) had a relative content of PA and elemicin of less than 50%, the predominant compounds were still PA (38.0%~49.8) and elemicin (38.1% and 46.2%). Furthermore, the volatile compounds with a relative content ratio of over 10% in at least one accession were 1-octen-3-ol (15.0–20.9% in three accessions), 2,4-hexadienal (13.9% in one), dill apiol (13.3% in one), methyl benzoate (11.5–19.9% in three), isoegomaketone (16.2% in one), PK (11.6% and 38.1% in two), linalool (13.3% in one), (Z,E)-α-farnesene (10.3–21.0% in seven), eugenol (12.0% in one), and caryophyllene (10.4% in one). The accession PF21-012, which is clustered with CF accessions using different clustering methods, contained high ratios of several compounds, including PK (38.1%), isoegomaketone (16.2%), (Z,E)-α-farnesene (15.6%), D-limonene (9.3%), caryophyllene (5.3%), linalool (4.2%), and 1-octen-3-ol (3.7%) (Additional file 2: Table S1). Therefore, this accession can be classified as PK with isoegomaketone type.

Few studies have been conducted to identify the volatile components of WF. This study confirmed the presence of volatile compounds in WF, and among the 41 compounds detected in WC two compounds (elemicin and α-terpineol) were not detected in WF (Table 2).

WF is intermediate in morphology compared with CF and WC [3]. It has green leaves and stems like CF and smaller and harder seeds like WC. When comparing the volatile compounds among the three types of *Perilla*, only two compounds were absent in WF, whereas seven compounds among all the volatile compounds were absent in CF (Table 2). According to ANOVA, 27 volatile compounds showed significant differences in their content between WF and WC, and six volatile compounds exhibited significant differences in their content between WF and CF (Table 2). These results suggest that WF still shows intermediate characteristics in the composition of volatile compounds, and it is difficult to distinguish it from CF and WC based on both morphological characteristics and volatile compounds. This tendency can also be observed in the results of PCA, K-means clustering, HCA, and heatmap analyses, which showed a clear distinction between CF and WC (Table 3; Figs. 3 and 4, and 5). However, WF accessions were not clearly separated from CF or WC, i.e., some WF accessions (PF20-095, PF20-106, PF20-085, and PF21-010) were grouped with other WC accessions (Table 3; Figs. 3 and 4, and 5) while the remaining 16 WF accessions were grouped together with the CF accessions.

In this study, WF accessions exhibited greater variability in volatile compounds compared with CF accessions, while showing less variability compared with WC accessions (Fig. 1, Additional file 2: Tables S1 and S2). Seventeen accessions (PF20-005, PF20-011, and PF20-026 for PK type with isoegomaketone; PF20-137, PF20-024, PF20-039, PF20-083, PF20-142, PF20-098, PF20-153, PF20-115, PF20-121, PF20-103, PF20-149, PF19-133, and PF20-112 for PK type with no isoegomaketone; PF20-095 for PP of myristicin) had a high relative content of PK (52.3%~89.8%) and myristicin (77.5%) with a ratio of over 50%. Although three accessions (PF20-106 and PF21-010 for PP type of dill apiol; PF20-085 for PP type of myristicin) had a relative content of PA and elemicin of less than 50%, the predominant compounds were dill apiol (36.7% and 46.8) and myristicin (46.9%). Furthermore, the volatile compounds with a relative content ratio of over 10% in at least one accession were 1-octen-3-ol (11.1 in one accession), dill apiol (22.7% in one), methyl salicylate (13.6% in one), isoegomaketone (12.1%~31.2% in three), (E)-β-farnesene (11.0% in one), and (Z,E)-α-farnesene (11.6% in one). WC and WF are not cultivated by farmers in South Korea, and they are often found growing around farmers' houses and fields of CF [3, 4]. Natural hybridization between weedy and cultivated types and introgression of many useful genes makes them adapted to natural growing conditions [40]. These complex content and composition patterns in WF may be the result of natural hybridization between WF and CF or WF and WC, which might have originated from multiple sources. Currently, there are no precise reports on the rate of natural hybridization among cultivated types of *Perilla* and its weedy types. However, previous studies have recently reported successful artificial hybridization between var. *frutescens* and var. *crispa* [12, 41, 42].

The composition of compounds can vary depending on the plant part (leaves, stems, seeds), treatment (fresh or dried), growth stage (seedling or mature), geographical location, etc. [17, 18, 43, 44]. The volatile compounds present in the fresh leaves at the heading period of CF, WF, and WC were collected from various regions (northern Gyeonggi-do, central Chungcheongnam-do, southern Gyeongsangnam-do, Jeollabuk-do, and Jeollanam-do) of South Korea. This study conducted a correlation test between the 41 volatile compounds and the collecting regions, and this revealed statistically significant differences in a few compounds (isoegomaketone with $r^2 = -0.350$ at $p < 0.01$, 1-octen-3-ol with $r^2 = 0.261$, and methyl benzoate with $r^2 = 0.220$ of at $p < 0.05$) (Data not shown). Although these compounds have relatively low correlation coefficient values, three out of four accessions (PF21-025, PF21-027, and PF21-028) collected from the northern part of South Korea contained isoegomaketone (12.1%~31.2%) (Table 1, Additional file 2: Table S1). However, through further research analyzing the accessions from excluded regions (such as Gangwon-do and other cities in the same area as those in this study), it is expected that more distinct regional patterns can be revealed. A previous study of CF and CC in China detected volatile compounds at the microgreen (seedling) stage [17]. Although this previous study was performed using only two *Perilla* cultivars, it showed that Chinese CC with purple leaves has D-limonene as the main compound with low levels of PA, while Chinese CF with green leaves has 2-hexanoylfuran as the main compound with a high ratio of perillene. Another previous study [18] confirmed PA and limonene as the main compounds in CC (Zisu), while PK was identified as the main compound in CF (Baisu) in China. These findings are similar to the results of our study on volatile compounds. The results indicate that differences and similarities could be attributed to variations in collecting region, growth stage, number of accessions, customer preferences, and cultivation environment [17, 18, 43, 44].

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13765-023-00801-6.

Additional file 1: Figure S1. The geographicdistribution of 80accessionsof *Perilla* crop collected from SouthKorea.

Additional file 2: Table S1. *Perilla* accessions from different areasof South Korea used for volatile compounds profiling. **Table S2.**Composition and comparison of volatile compounds of three types of *Perilla* accessions. **Table S3.** K-mean clustering inK=3 of *Perilla* accessions usingvolatile compounds.

Acknowledgements
We are thankful to all the institutions and people who helped with this study.

Author contributions
Funding acquisition, conceptualization, supervisionand manuscript revision: K.J.S., S.L.; methodology, data analysis andoriginal-draft preparation: S.J.J., H.P.; Methodology: J.C.; fundingacquisition and conceptualization: J.S., J.K.L.

Funding
Thisstudy was supported by the Cooperative Research Program for AgricultureScience & Technology Development (project no. PJ01422701), RuralDevelopment Administration, Republic of Korea.

Availability of data and materials
The datasets used and analyzed in this study are available from the corresponding author upon reasonable request.

Declarations

Competing interests
The authors declare that there are no financial or other relationships that mightlead to a conflict of interest of the present article. All authors have reviewed thefinal version of the manuscript and approved it for publication.

Received: 12 June 2023 Accepted: 8 July 2023
Published online: 21 July 2023

References

1. Makino T (1961) Makino's new illustrated flora of Japan. Hokuryukan, Tokyo. (in Japanese)
2. Li HL (1969) The vegetables of ancient China. Econ Bot 23:235–260. http://www.jstor.org/stable/4253062
3. Lee JK, Ohnishi O (2001) Geographic differentiation of morphological characters among *Perilla* crops and their weedy types in East Asia. Breed Sci 51:247–255. https://doi.org/10.1270/jsbbs.51.247
4. Lee JK, Ohnishi O (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. https://doi.org/10.1023/A:1022951002271
5. Nitta M (2001) Origin of *Perilla* crops and their weedy type. Ph.D. Thesis, Kyoto University, Kyoto, p 78
6. Nitta M, Lee JK, Ohnishi O (2003) Asian *Perilla* crops and their weedy forms: their cultivation, utilization and genetic relationships. Econ Bot 57:245–253
7. Nitta M, Lee JK, Kobayashi H, Liu D, Nagamine T (2005) Diversification of multipurpose plant, *Perilla frutescens*. Genet Resour Crop Evol 52:663–670. https://doi.org/10.1007/s10722-003-6013-9
8. Lee JK, Nitta M, Kim NS, Park CH, Yoon KM, Shin YB, Ohnishi S (2002) Genetic diversity of *Perilla* and related weedy types in Korea determined by AFLP analyses. Crop Sci 42:2161–2166. https://doi.org/10.2135/cropsci2002.2161
9. Nagai I (1935) On "Shiso" and "Egoma. Agric Hortic 10:2265–2273 (in Japanese)
10. Yamane Y (1950) Cytogenetic studies on the genus *Perilla* and Coleus I. chromosome number. Jpn Genet 25:220 ((**in Japanese**))
11. Honda G, Yuba A, Kojima T, Tabata M (1994) Chemotaxonomic and cytogenetic studies on *Perilla frutescens* var. *Citriodora* (Lemon Egoma). Nat Med 48:185–190
12. Kim JY, Sa KJ, Ha YJ, Lee JK (2021) Genetic variation and association mapping in F_2 population of *Perilla* crop (*Perilla frutescens* L.) using new developed Perilla SSR markers. Euphytica 217:135. https://doi.org/10.1007/s10681-021-02867-z
13. Ahmed HM, Tavaszi-Sarosi S (2019) Identification and quantification of essential oil content and composition, total polyphenols and antioxidant capacity of *Perilla frutescens* (L.) Britt. Food Chem 275:730–738. https://doi.org/10.1016/j.foodchem.2018.09.155
14. Koul O, Walia S, Dhaliwal GS (2008) Essential oils as green pesticides: potential and constraints. Biopestic Int 4:63–84
15. Mihaylova D, Popova A, Vrancheva R, Dincheva I (2022) HS-SPME-GC–MS volatile profile characterization of peach (*Prunus persica* L. Batsch) varieties grown in the Eastern Balkan Peninsula. Plants 11:166. https://doi.org/10.3390/plants11020166
16. Hou T, Netala VR, Zhang H, Xing Y, Li H, Zhang Z (2022) *Perilla frutescens*: a rich source of pharmacological active compounds. Molecules 27:3578. https://doi.org/10.3390/molecules27113578
17. Dimita R, Min Allah S, Luvisi A, Greco D, De Bellis L, Accogli R, Mininni C, Negro C (2022) Volatile compounds and total phenolic content of *Perilla frutescens* at microgreens and mature stages. Horticulturae 8:71. https://doi.org/10.3390/horticulturae8010071
18. Huang B, Lei Y, Tang Y, Zhang J, Qin L, Liu J (2011) Comparison of HS-SPME with hydrodistillation and SFE for the analysis of the volatile compounds of Zisu and Baisu, two varietal species of *Perilla frutescens* of chinese origin. Food Chem 125:268–275. https://doi.org/10.1016/j.foodchem.2010.08.043
19. Chen F, Liu S, Zhao Z, Gao W, Ma Y, Wang X, Yan S, Luo D (2020) Ultrasound pretreatment combined with microwave-assisted hydrodistillation of essential oils from *Perilla frutescens* (L.) Britt. Leaves and its chemical composition and biological activity. Ind Crops Prod 143:111908. https://doi.org/10.1016/j.indcrop.2019.111908
20. Koezuka Y, Honda G, Tabata M (1986) Genetic control of the chemical composition of volatile oils in *Perilla frutescens*. Phytochem 25:859–963. https://doi.org/10.1016/0031-9422(86)80017-6
21. Ito M, Toyoda M, Kamakura S, Honda G (2002) A new type of essential oil from *Perilla frutescens* from Thailand. J Essent Oil Res 14:416–419. https://doi.org/10.1080/10412905.2002.9699907
22. Nitta M, Kobayashi H, Ohnishi-Kameyama M, Nagamine T, Yoshida M (2006) Essential oil variation of cultivated and wild *Perilla* analyzed by GC/MS. Biochem Syst Ecol 34:25–37. https://doi.org/10.1016/j.bse.2005.05.006
23. Choi HS, Min KC (2003) Headspace analysis of *Perilla frutescens* var. Acuta Kudo by solid-phase microextraction using diverse fibers. Food Sci Biotechnol 12:409–414
24. Choi HS (2004) Characteristic odor components of *Perilla frutescens* var. Acuta kudo oil by aroma extract dilution analysis. Food Sci Biotechnol 13:279–284
25. Tian J, Zeng X, Zhang S, Wang Y, Zhang P, Lü A, Peng X (2014) Regional variation in components and antioxidant and antifungal activities of *Perilla frutescens* essential oils in China. Ind Crops Prod 59:69–79. https://doi.org/10.1016/j.indcrop.2014.04.048
26. Yu H, Qiu JF, Ma LJ, Hu YJ, Li P, Wan JB (2017) Phytochemical and phytopharmacological review of *Perilla frutescens* L. (Labiatae), a traditional edible-medicinal herb in China. Food Chem Toxicol 108:375–391. https://doi.org/10.1016/j.fct.2016.11.023
27. Jang HJ, Park JY, Kim YT (1991) Volatile components of *Perilla* folium. Korean J Food Sci Technol 23:129–132
28. Yu H, Kosuna K, Haga M (1997) The Genus Perilla. Netherlands Overseas Publishers' Association
29. Seo WH, Baek HH (2009) Characteristic aroma-active compound of korean perilla (*Perilla frutescens* Britton) leaf. J Agric Food Chem 57:11537–11542. https://doi.org/10.1021/jf902669d
30. Ahmed HM, Adel MAA (2020) Exploring natural essential oil components and antibacterial activity of solvent extracts from twelve *Perilla frutescens* L. genotypes. Arab J Chem 10:7390–7402. https://doi.org/10.1016/j.arabjc.2020.08.016
31. Pang Z, Chong J, Zhou G, Morais D, Chang L, Barrette M, Gauthier C, Jacques PE, Li S, Xia J (2021) MetaboAnalyst 5.0: narrowing the gap between raw spectra and functional insights. Nucleic Acids Res 49:W388–W396. https://doi.org/10.1093/nar/gkab382
32. Banno N, Akihisa T, Tokuda H, Yasukawa K, Higashihara H, Ukiya M, Watanabe K, Kimura Y, Hasegawa J, Nishino H (2004) Triterpene acids from the leaves of *Perilla frutescens* and their anti-inflammatory and

antitumor-promoting effects. Biosci Biotechnol Biochem 68:85–90. https://doi.org/10.1271/bbb.68.85

33. Osakabe N, Yasuda A, Natsume M, Yoshikawa T (2004) Rosmarinic acid inhibits epidermal inflammatory responses: anticarcinogenic effect of *Perilla frutescens* extract in the murine two-stage skin model. Carcinogenesis 25:549–557. https://doi.org/10.1093/carcin/bgh034
34. Park HY, Nam MH, Lee HS, Jun W, Hendrich S, Lee KW (2010) Isolation of caffeic acid from *Perilla frutescens* and its role in enhancing γ-glutamylcysteine synthetase activity and glutathione level. Food Chem 119:724–730. https://doi.org/10.1016/j.foodchem.2009.07.020
35. Li HZ, Zhang ZJ, Hou TY, Li XJ, Chen T (2015) Optimization of ultrasound-assisted hexane extraction of perilla oil using response surface methodology. Ind Crops Prod 76:18–24. https://doi.org/10.1016/j.indcrop.2015.06.021
36. Chang X, Xu H, Wang D, Feng Y, Zhan P (2013) Recovery of perillaldehyde from hydrodistillation condensate of *Perilla frutescens* (L.) Britton var. *Crispa* F. *viridis* leaves using macroporous resin adsorption. Sep Sci Technol 48:1226–1233. https://doi.org/10.1080/01496395.2012.726686
37. Kulkarni PS, Brazinha C, Afonso CAM, Crespo JG (2010) Selective extraction of natural products with benign solvents and recovery by organophilic pervaporation: fractionation of d-limonene from orange peels. Green Chem 12:1990–1994. https://doi.org/10.1039/C0GC00339E
38. Laureati M, Buratti S, Bassoli A, Borgonovo G, Pagliarini E (2010) Discrimination and characterisation of three cultivars of *Perilla frutescens* by means of sensory descriptors and electronic nose and tongue analysis. Food Res Int 43:959–964. https://doi.org/10.1016/j.foodres.2010.01.024
39. Ha TJ, Lee MH, Lee JH (2015) Comparison of antioxidant activities and volatile components using GC/MS from leaves of korean purple perilla (*Perilla frutescens*) grown in a greenhouse. Food Sci Biotechnol 24:1979–1986. https://doi.org/10.1007/s10068-015-0261-2
40. Cho YC, Chung TY, Suh HS (1995) Genetic characteristics of korean weedy rice (*Oryza sativa* L.) by RFLP analysis. Euphytica 86:103–110. https://doi.org/10.1007/BF00022015
41. Lim SE, Sa KJ, Ha YJ, Lee JK (2019) Genetic analysis of F2 population derived from the cross between *Perilla frutescens* var. *frutescens* and var. *crispa*. Korean J Breed Sci 51:184–189
42. Lim SE, Sa KJ, Lee JK (2021) Bulk segregant analysis identifies SSR markers associated with leaf-and seed related traits in *Perilla* crop (*Perilla frutescens* L). Genes Genom 43:323–332. https://doi.org/10.1007/s13258-021-01056-5
43. Liu J, Wan Y, Zhao Z, Chen H (2013) Determination of the content of rosmarinic acid by HPLC and analytical comparison of volatile constituents by GC-MS in different parts of *Perilla frutescens* (L.) Britt. Chem Cent J 7:61. https://doi.org/10.1186/1752-153X-7-61
44. Xie J, Li X, Li W, Ding H, Yin J, Bie S, Li F, Tian C, Han L, Yang W, Song X, Yu H, Li Z (2023) Characterization of the key volatile organic components of different parts of fresh and dried *Perilla frutescens* based on headspace-gas chromatography-ion mobility spectrometry and headspace solid phase microextraction-gas chromatography-mass spectrometry. Arab J Chem 16:104867. https://doi.org/10.1016/j.arabjc.2023.104867

Publisher's Note