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Comparison of Analytical Methods for Volatile Flavor Compounds in Leaf of *Perilla frutescens*

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ABSTRACT

Volatile flavor compounds from perilla leaves were extracted and analyzed with different methods, headspace analysis(HS), simultaneous steam distillation and extraction (SDE), and solvent extraction (SE), and to compare their efficiencies for quick analysis. Over 30 volatile compounds were isolated and 28 compounds were identified by GC/MSD. Major compound was perillaketone showing the compositions of which were 92% in SDE method, 86% in headspace analysis, and 62% in solvent extraction method. For quick evaluation of leaf flavor in perilla, it was desirable because the headspace analysis method had a shorter analyzing time and smaller sample amount than the other methods.

Keywords : *Perilla frutescens*, volatile flavor compounds, analytical methods.

Perilla (*Perilla frutescens*) is an annual herbal crop belonging to Labiatae, and has been cultivated for a long time as a traditional oil crop in Korea. Its seeds have been used as oil or tea and its leaves have been increasingly consumed as a vegetable in winter.

Kwak (1994) reported that the seeds contained 40.3 g/100 g seeds of oil and 61.5% of linolenic acid of fatty acid in Korean native perilla collections. Perilla leaves had perillaketone as major volatile compounds in Korean varieties and emited an unique flavor (Lim et al., 1994). The leaves contained 0.26% essential oil before flowering, in addition to vitamins and inorganic compounds such as Ca, Mg, etc. (Kwak and Lee, 1995). The steam distillate of the green leaf of perilla had broad antimicrobial activity and mainly consisted of perillaldehyde, limonene, β-caryophyllene, α-bergamotene, and linalool (Kang et al., 1992).

Chemotypes, on the basis of essential oil type of perilla leaf (*Perilla frutescens*) of Japanese varieties were grouped into 5 types, perillaldehyde (PA), perillaketone (PK), elsholtziaketone (EK), citral (C), phenylpropanoid (PP) (Koezuka et al., 1984). It was reported that their volatile components were controlled by major genes based on genetic analysis of their chemotypes (Koezuka et al., 1986a, 1986b, 1986c; Nishizawa et al., 1991).

Pino et al. (1996) reported that the yield of volatile compounds from *Coleus aromaticus* leaf reached at 0.55% in steam distillation, 6.52% from hexane extraction, and 1.40% by supercritical CO_2 extraction, which its oil content differed with different analytical procedures. In addition, the aromatic difference of the extracts was quite noticeable and could be attributed to qualitative and quantitative differences of the components.

Research on perilla in Korea has been focused on improvement of seed oil quality as well as higher oil content, but less composition of linolenic acid. Perilla, however, has been recently recognized as an important leafy vegetable crop. The results on volatile flavor and their functions have been reported (Chung et al., 1998), and reviewed perilla volatile components (Lee et al., 1998). So the setup of the proper analytical technique is required for the improvement of volatile components of perilla leaves.

The experiments aim was to understand changes of volatile compounds in perilla leaves according to different analytical methods, headspace method (HS), simutaneous steam distillation and extraction (SDE), and solvent extraction (SE). Consequently, a quick evaluation system for the selection of perilla lines with diverse volatile components was set up.

MATERIALS AND METHODS

We used leaves of perilla cv. Chubu kept in a deep freezer immediately after harvest. For extraction and analysis of oil and volatile compounds, simultaneous steam distillation and extraction apparatus, headspace autosampler (Tekmar 7000, Tekmar-Dohrmann Co., USA), and gas chromatograph/ mass spectrometer (GC/MSD; HP6890/HP5973, Hewlett Packard Co., USA) were used in the Instrument Analysis Laboratory, National Crop Experiment Station, RDA.

In headspace method (HS), we used 1 completely matured leaf (around 2 g). This leaf was put into a 22 ml autosampler bottle, then sealed and loaded to the headspace autosampler. The sample in the bottle was equilibrated for 15 min. at 65℃, and 1 ml of gas volatized from the sample was injected automatically into gas chromatography equipped with a 50 m Ultral-1 capillary column (Hewlett-Packard Co.,

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USA) and a flame ionization detector. Volatile compounds in the gas mixture were separated through the GC column, and a spectrum of each compound obtained by Mass Selective Detector in GC/MSD, was identified using HP Chemstation software and mass spectrum libraries (Wiley 275 and NBS). Analytical conditions of Headsapce Autosampler and GC/MSD were the same as Table 1 and Table 2.

In simultaneous steam distillation and extraction (SDE) method for oil extraction, SDE apparatus was used to distill and extract essential oil from 200 g of fresh leaves for 3hours. Extraction solvent was a 1:1 mixture of diethyl ether and pentane. After the extracted oil fraction was transferred to a round flask and stored in a freezer for 6 hrs, this fraction was separated from water fraction. For dehydration, anhydrous sodium sulfate(Na_2SO_4) was added to the extract and left overnight. Solvent in oil extract was evaporated in vacuum at less than 30℃ and finally under N_2 stream. The residual essential oil was weighed for the oil content, and then analyzed under analytical condition of GC/MSD (Table 2).

Finally, n-hexane was used as an extraction solvent in the solvent extraction method (SE). 100 g of fresh sample was cut into pieces and transferred to flask. 400 ml n-hexane was added to the sample, placed and extracted at 4℃ for 24 hours. The extracted samples were filtrated by Whatman No. 1 filter paper and dehydrated overnight using anhydrous sodium sulfate. The solvent was removed and the residual oil was analyzed using GC/MSD like in the SDE method.

Mass spectra of each peak on chromatogram were obtained and identified using Wiley 275 and NBS libraries and compared the retention time to references published previously.

We could compare and calculate the compositions of volatile compounds based on peak area from the above.

Table 1. Analytical condition of headspace autosampler for determination of volatile components in perilla leaf.

Instrument	Tekmar 7000
Platen temperature	65℃
Sample equilibration time	15 min.
Vial size	22 ml
Mixing time	1 min.
Sample loop temperature	70℃
Transfer line temperature	70℃

Table 2. Analytical condition of GC/MSD (Gas chromatograph/Mass spectrometer) for volatile components.

Instrument	HP 6890 GC/ HP 5973 MSD
Column	Ultra-1 (crosslinked methyl siloxane, 50 m×0.2 mm×0.33 m)
Injector temperature	250℃
Oven temp. program	50℃ (5 min.)→230℃ (10 min.) at 3℃/min.
Auxiliary temperature	280℃
Carrier gas	Helium
Flow rate	0.8 ml/min.
Split ratio	1 : 40
Ionization mode	Electron Impact (70 eV)
MS source temperature	230℃

RESULTS AND DISCUSSION

Content of the oil extracted by SDE method in perilla cv. Chubu leaves was 0.47% based on their dry weight. We analyzed the oil extract using GC/MSD and obtained a total ion chromatography (TIC) as

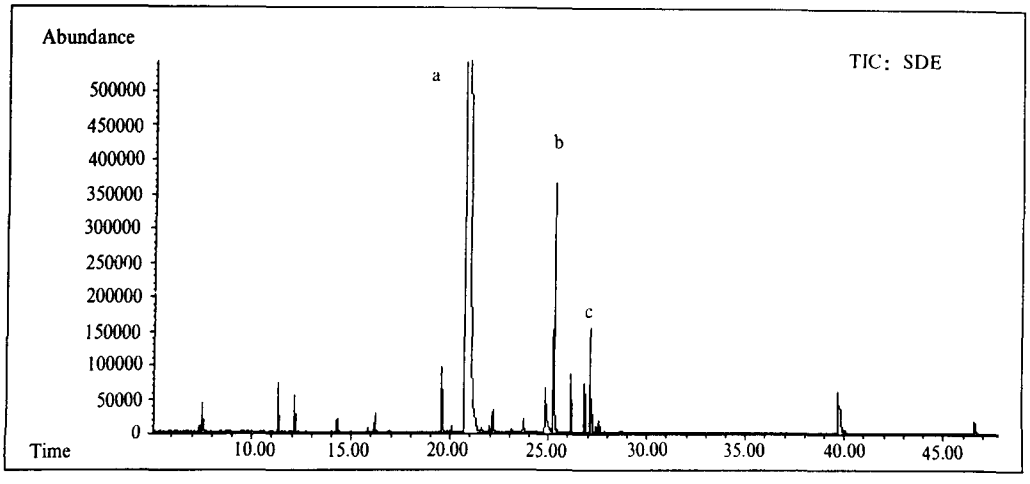

Fig. 1. Total ion chromatogram of volatile compounds of SDE extract from perilla leaves. *a, perillaketone; b, β-caryophyllene and c, α-farnesene.

shown in Fig. 1. Major volatile compounds were perillaketone, and β-caryophyllene as sesquiterpene, and cv. Chubu could be grouped into PK (perillaketone) chemotype.

TIC from HS to separate the volatile compounds emitted from fresh leaves was shown in Fig. 2. Major compound was perillaketone like in SDE. Pattern of chromatogram in HS was simpler than in SDE, meaning that volatile compounds extracted and volatility of each compound were different according to extraction methods. Actually on proceeding HS, water vapor gave rise to a serious problem in the column because water in fresh leaves changed it when the sample was equilibrated in the plate of headspace autosampler. We had to freeze-dry the samples and compared analyzed data from dry and fresh conditions, not to make the difference significantly. So additional equipment for water or freeze-drying with fresh samples was recommended.

In SE using n-hexane as extracting solvent, major compound was also perillaketone like SDE and HS. Dominant non-volatile compounds dissolved in n-hexane in addition to volatile compounds appeared in TIC (Fig. 3), and we estimated those compounds were long chain hydrocarbons with over 21 of carbon atoms. Although this SE method seemed to be possible to group the chemotypes of plants, it was not appropriate for analysis of volatile flavor compounds. n-Hexane extract contained relatively more non-volatile compounds contaminating a column and injector and leading to imprecise results. So we needed to purify solvent extract through SDE or col umn chr-

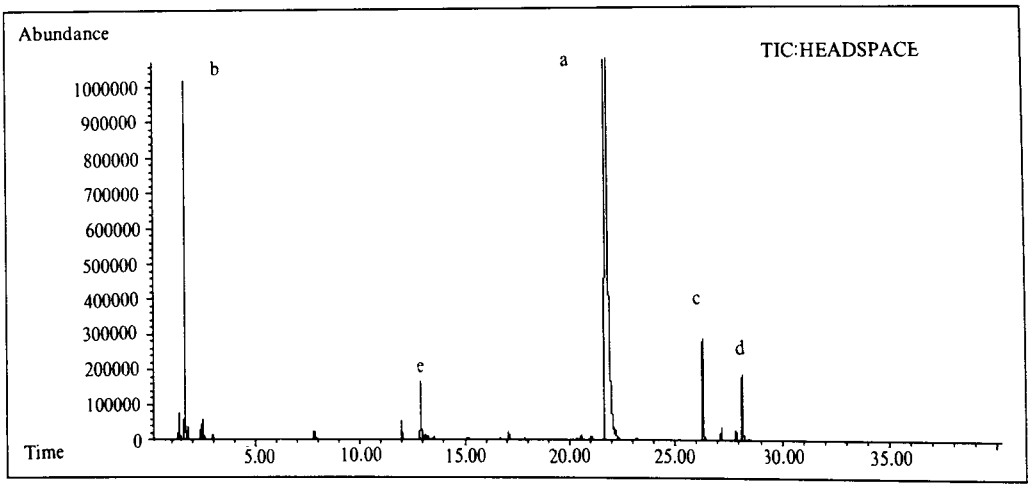

Fig. 2. Total ion chromatogram of volatile compounds by headspace analysis from perilla leaves.
*** a, perillaketone; b, methyl sulfide; c, β-caryophyllene; d, γ-farnesene and e, 1-octen-3-ol.**

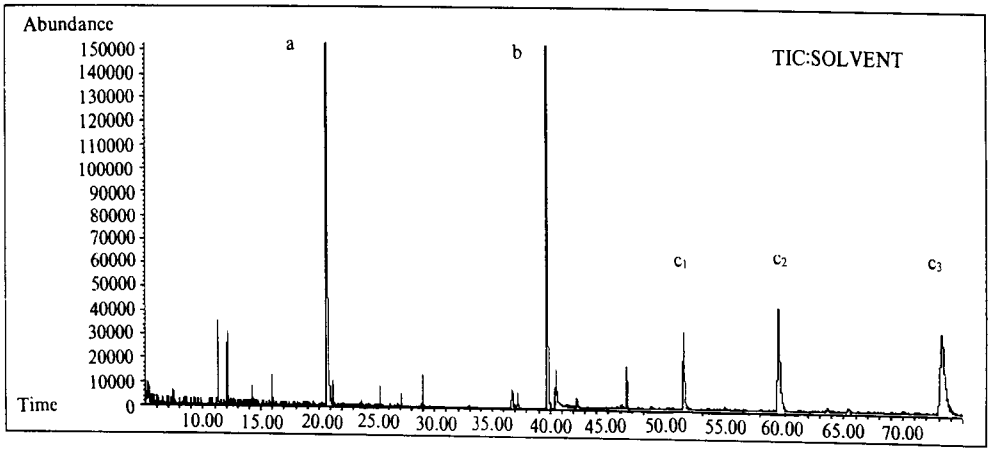

Fig. 3. Total ion chromatogram of volatile compounds of solvent (hexane) extracts from perilla leaves.
*** a, perillaketone; b, ethyl linolate; c1, c2, and c3, hydrocarbons.**

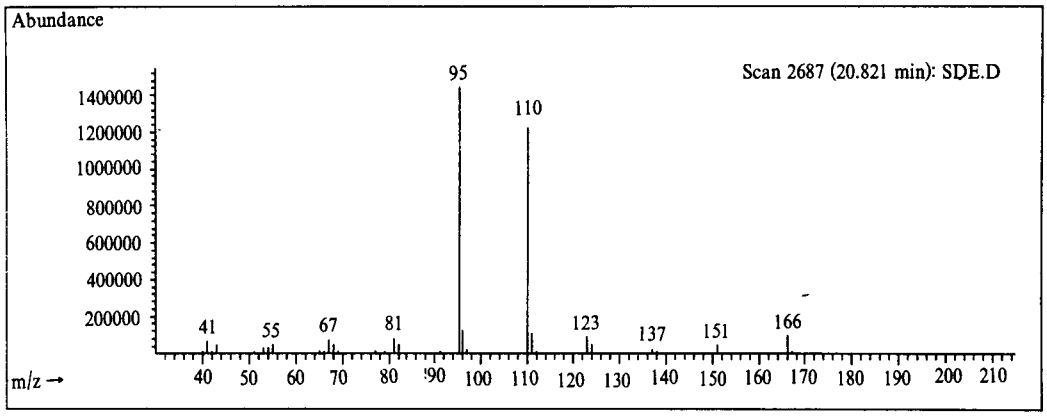

Fig. 4. Mass spectrum of perillaketone, major volatile compound from perilla cv. Chubu leaves.

Table 3. Compositions and peak areas(%) of volatile compounds identified in different analytical methods, headspace analysis (HS), simultaneous steam distillation and extraction (SDE) and solvent extraction (SE) in perilla leaves.

Peak No.	Compounds	Peak area (%)		
		HS	SDE	SE
1	Ethyl alcohol	0.35	-	-
2	Methyl sulfide	6.01	-	-
3	Isobutyl aldehyde	0.28	-	-
4	2-Methyl butanal	0.26	-	-
5	2-Ethyl furan	0.33	-	-
6	Propanoic acid	trace	-	-
7	trans-2-Hexenal	trace	0.05	0.20
8	cis-3-Hexenol	0.23	0.24	0.26
9	Benzaldehyde	0.43	0.38	1.47
10	1-Octen-3-ol	1.22	0.27	1.13
11	Amylethyl ketone	0.08	-	-
12	β-Myrcene	0.06	-	-
13	tert-Pentyl benzene	-	0.03	-
14	3-Octanol	0.07	0.02	-
15	Phenylacetaldehyde	0.03	0.10	0.20
16	Unknown	0.03	0.57	-
17	Linalool	0.16	0.14	-
18	Unknown	0.02	0.05	-
19	Perillaketone	85.93	92.33	62.17
20	Terpinolene	-	0.27	-
21	α-Terpinene	-	0.02	-
22	3,4-Dichloroaniline	-	0.78	-
23	β-Caryophyllene	2.07	1.97	0.18
24	α-Humulene	0.28	0.48	trace
25	Germacrene D	0.20	0.38	trace
26	α-Farnesene	1.36	0.78	trace
27	Bicyclogermacrene	0.04	0.23	trace
28	Methyl benzoate	-	-	0.46
29	Ethyl linolate	-	-	8.35
30	Hydrocarbons (over C21)	-	-	22.91

omatography for analysis of volatile flavor compounds.

We compared the compositions of volatile compounds using relative peak area and retention time of each peak according to the analytical methods(Table 3). Because of stronger volatility at low temperature, several highly volatized compounds were not shown in SDE, but 6.0% methyl sulfide among them was contained in HS.

Perilla cv. Chubu was recognized as perillaketone (Fig. 4) group into PK chemotype. Compositions of the compound in SDE, HS and SE were 92%, 86% and 62%, respectively. So three methods might be used to evaluate the chemotypes of plants based on volatile compounds because 28 compounds among over 30 volatile compounds isolated were identified by GC/MSD.

We also think that the best method to group the chemotypes and evaluate the flavor quality is SDE, but it takes a relatively long time and needs much more (over 100 g) samples. Therefore we suggest that HS is a relatively more appropriate method to evaluate the chemotypes with a small amount of sample (one matured leaf) and to screen mutants or pilot lines quickly in the volatile condition closed to fresh sample.

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