Korean J. Medicinal Crop Sci. 12(2) : 97 – 101 (2004)

Analysis of Volatile Oil Components and Identification of Chemotypes in Jaso (*Perilla frutescens*) Collected in Korea

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ABSTRACT : Volatile oil components were analysed in *Perilla frutescens* accessions collected from different regions in South Korea and identified chemotypes based on the major volatile oil components. Major components out of 30 compounds identified were limonene, perillaldehyde, perillaketone, isoegomaketone, beta-caryophyllene, beta-farnesene, myristicin, and dillapiole. *P. frutescens* collections were classified into four chemotypes : PA type (57.7% limonene and 19.8% perillaldehyde), PK type (89.8% perillaketone), ST type (82.4% sesquiterpene, as 54.5% beta-caryophyllene and 27.9% beta-farnesene) and PP type (40.3% phenylpropenes as 13.6% myristicin and 26.7% dillapiole) and 37.8% sesquiterpenes. The majorities of *P. frutescens* collections in this study belong to PA type (41.9%) and PK type(38.8%).

Key words : *Perilla frutescens,* local collections, volatile oils, chemotypes

INTRODUCTION

Within the genus *Perilla*, Jaso is different from Deulkkae in the form of leaf and stem, and flower color. Jaso is divided into purple perilla (*Perilla frutescens* Britton var. *acuta* Kudo), ruffle-leaved purple perilla (*P. frutescens* Britton var. *crispa* Hand.-Mazz. *f. atropurpurea*), wild perilla (*P. frutescens* Britton var. *viridis* Makino) and upper-green purple perilla (*P. fruteseens* Britton var. *japonica* Hara. for *discolor* Makino) on the basis of morphological characteristics (Han *et al.*, 2000). Purple perilla has the purple leaf, stem and flower. Ruffle-leaved purple perilla has the ruffled form of leaf and purple flower. Wild perilla has the green leaf and purple flower. Upper-green purple perilla has the only one-sided purple leaf.

The leaves of Jaso are have been used as a traditional Chinese herb medicine for cold and cough remedies, and promoting digestion (Duke, 1988). This fast-growing herb is used in a wide variety of applications including foods, food coloring, and flavoring. In Japan, genus Perilla has been classified into five major chemotypes according to the main components of their volatile oils; perillaldehyde, perillaketone, elsholtziaketone, citral and phenylpropanoid type (Koezuke *et al.*, 1984).

Introduction of anthocyanin pigments from purple perilla to Deulkkae (green perilla) has been studied by crossing them even though volatile oil components in Jaso were not analysed extensively. To increase the utility value of Jaso as an aromatic crop as well as a source of anthocyanins, we compared the characteristics of volatile components in the Jaso collected from the different regions of South Korea and identified different chemotypes with them.

MATERIALS AND METHODS

Plant materials

Jaso was collected from 13 regions (Table 1). Wild perilla and ruffle-leaved purple perilla were collected at Milyang, and upper-green purple perilla were

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Received February 16, 2004 / Accepted Aprill 19, 2004

Table 1. Collection sites of *P. frutescens* in Korea

Variety	Collection site	Symbol
P. f. Brit. var. *viridis* Makino	Milyang (36)†	a
P. f. Brit. var. *crispa* Hand.-Mazz. *f. atropurpurea*	Milyang (40)	b
P. f. Brit. var. *japonia* Hara. for. *discolor* Makino	Hongcheon (32)	c
	Milyang (32)	d
	Sacheon (32)	e
P. f. Brit. var. *acuta* Kudo	Cheongyang (20)	f
	Dangjin (19)	g
	Inje (18)	h
	Milyang (19)	i
	Naju (20)	j
	Suwon (17)	k
	Pocheon (17)	l
	Yangpyeong (18)	m

† The numbers in the parentheses indicate the numbers of analyzed samples.

collected at Hongcheon, Milyang and Sacheon. Purple perilla was collected at Cheongyang, Dangjin, Inje, Milyang, Naju, Suwon, Pocheon, and Yangpyeong. Individual plants from these 13 accessions were harvested at flowering stage for the analysis of volatile components after greenhouse-growing at the College Experimental Farm, Seoul National University, Suwon.

Headspace sample collection

From the individual plant samples harvested at each pot in experimental greenhouse, two to three grams of leaves were weighed and placed into 22 ㎖ glass bottles without damaging the tissue. The leaves were immediately freeze-dried and sealed with teflon-coated septa and aluminum seals.

GC-MS Analysis

Volatiles were analyzed on the equilibrium headspace autosampler (Tekmar 7000) connected with gas chromatography/mass spectrometry (Hewlett-Packard 6890/5973) equipped with "chemstation" software . A fused-silica HP-5 capillary column (1.0 film thickness, 0.25 ㎜ (id) x 30 m, Hewlett-Packard, USA) was used. Carrier gas was helium and flow rate was 1.0 ㎖/min., and the injector and detector temperatures were set to 250 and 280℃, respectively. The oven temperature was held isothermal at 80℃ for 3 min., and then, programmed to increase to 230℃ at 5℃/min. Identification of the headspace volatile compounds of individual plants within each population was performed by comparison of retention time and mass spectrum of samples with those of standards and Wiley 275 library (Wiley, USA).

RESULTS AND DISCUSSION

Headspace analysis was performed using the leaves collected at flowering stage. Thirty volatile components were identified (Table 2). The composition and contents of volatile components were varied among collection sites and individual plant. Components which average contents was above 1% out of the 30 components were alpha-pinene, 1-beta-pinene, limonene, perillaketone, perillaldehyde, isoegomaketone, beta-caryophyllene, beta-farnesene, myristicin, and dillapiole. Out of the above ten components, limonene, perillaldehyde, perillaketone, isoegomaketone, beta-caryophyllene, beta-farnesene, myristicin, and dillapiole were the major volatile components. Koezuka *et al.* (1984) reported that the major components of volatile oil from *P. frutescens* in Japan were variable and grouped into five chemotypes according to the contents of major components: perillaldehyde, elshotziaketone,

Table 2. The contents of volatile components in leaf of *Perilla frutescens* by headspace analysis.

	Mean of GC area (%)													Sig.†
	a	b	c	d	e	f	g	h	i	j	k	l	m	
1	0.43	0.41	1.15	1.97	4.56	2.12	-	-	1.34	1.29	-	1.59	5.35	***
2	0.06	0.16	-	0.18	-	-	-	4.77	0.06	0.01	0.39	-	-	***
3	0.08	0.14	-	0.44	0.94	0.24	-	-	0.25	0.23	-	0.38	1.51	***
4	0.10	0.12	-	0.42	-	-	-	-	0.43	0.05	0.26	-	0.07	ns
5	0.40	0.36	0.78	1.90	3.68	1.45	-	0.03	1.27	1.39	-	2.23	3.78	***
6	0.05	0.05	-	0.22	0.51	0.06	-	-	0.17	0.14	-	0.23	0.54	***
7	8.35	5.98	8.64	33.45	53.23	34.65	7.59	-	22.74	39.14	-	89.22	44.92	***
8	0.04	0.04	-	0.23	0.60	0.05	-	-	0.16	0.08	-	0.16	0.53	***
9	0.03	0.03	-	0.11	0.20	-	-	-	0.09	0.05	-	0.10	0.17	***
10	-	-	-	-	3.29	0.30	-	0.64	-	-	-	1.67	1.53	***
11	0.06	2.97	-	0.96	-	-	-	-	1.02	0.49	0.59	-	-	***
12	-	2.04	-	0.10	-	-	-	-	0.24	0.39	-	-	-	***
13	-	1.61	-	0.01	-	-	-	-	0.12	0.10	0.18	-	-	***
14	0.67	0.19	-	0.08	-	-	-	-	0.03	0.18	-	-	-	***
15	-	46.47	2.55	43.37	0.14	1.11	-	45.60	55.94	37.86	46.14	0.21	0.29	***
16	2.02	0.37	-	0.28	-	-	-	-	0.23	0.66	0.02	-	-	***
17	1.23	1.93	1.98	7.40	25.75	15.71	-	-	6.99	6.42	33.02	1.75	26.41	***
18	-	2.12	-	0.01	-	-	-	-	0.16	0.09	-	-	-	***
19	-	27.26	-	-	-	-	-	-	2.04	1.41	-	-	-	***
20	1.34	0.08	-	0.06	-	-	-	-	0.17	0.38	-	-	-	***
21	42.24	5.30	25.61	6.18	4.33	29.77	69.99	15.41	4.29	6.40	4.92	1.69	7.77	***
22	32.97	-	2.28	-	-	2.28	2.62	-	-	-	1.88	-	0.39	***
23	3.29	0.17	1.11	0.21	0.22	0.19	-	-	0.14	0.30	0.11	0.08	0.40	***
24	1.94	1.24	8.62	1.57	1.33	3.56	7.54	3.76	1.27	1.93	2.04	0.24	1.68	***
25	0.11	0.01	4.94	0.04	0.09	0.51	2.14	0.03	0.04	0.07	0.03	0.02	0.77	***
26	-	-	0.53	-	0.25	0.06	-	0.04	-	-	0.02	0.10	0.23	***
27	0.33	0.13	-	0.14	-	-	-	-	0.21	0.23	0.09	-	-	***
28	0.13	0.01	1.05	-	-	0.11	-	-	0.05	0.01	-	0.04	0.18	***
29	1.77	-	0.42	-	-	0.14	-	28.73	-	-	5.89	-	0.04	*
30	-	-	39.72	-	-	7.38	10.12	0.89	-	-	-	-	2.54	***
31	2.33	0.94	0.63	0.47	0.68	0.32	-	0.04	0.40	0.62	4.14	0.22	1.05	***

† Significance : *** $p<0.001$; ** $0.001 < p < 0.01$; * $0.001 < p < 0.5$; ns $p > 0.5$.

- not detected.

1:alpha-pinene, 2:1-octen-3-ol, 3:sabinene, 4:benzaldehyde, 5:1-beta-pinene, 6:2-beta-pinene, 7:limonene, 8:1,8-cineole, 9:terpinolene, 10:linalool, 11:delta-3-carene, 12:perillene, 13:camphenilone, 14:dodecane, 15:perillaketone, 16:tridecane, 17:perilaldehyde, 18:egomaketone, 19:isoegomaketone, 20:tetradecane, 21:beta-caryophyllene, 22:beta-farnesene, 23:alpha-humulene, 24:alpha-farnesene, 25:farnesene, 26:valencene, 27a:germacrene-d, 28:bicyclogermacrene, 29:myristicin, 30:dillapiole, 31:others

perillaketone, citral and phenylpropanoid types. Out of these five chemotype, elshotziaketone and citral types were not detected in Jaso grown in Korea, while sesquiterpenes such as beta-caryophyllene and beta-farnese which were not found in Japanese Jaso were detected as the major components in Cheongyang, Dangjin, and Milyang accessions.

Fig. 1 showed five major TIC (total ion chromatograph)

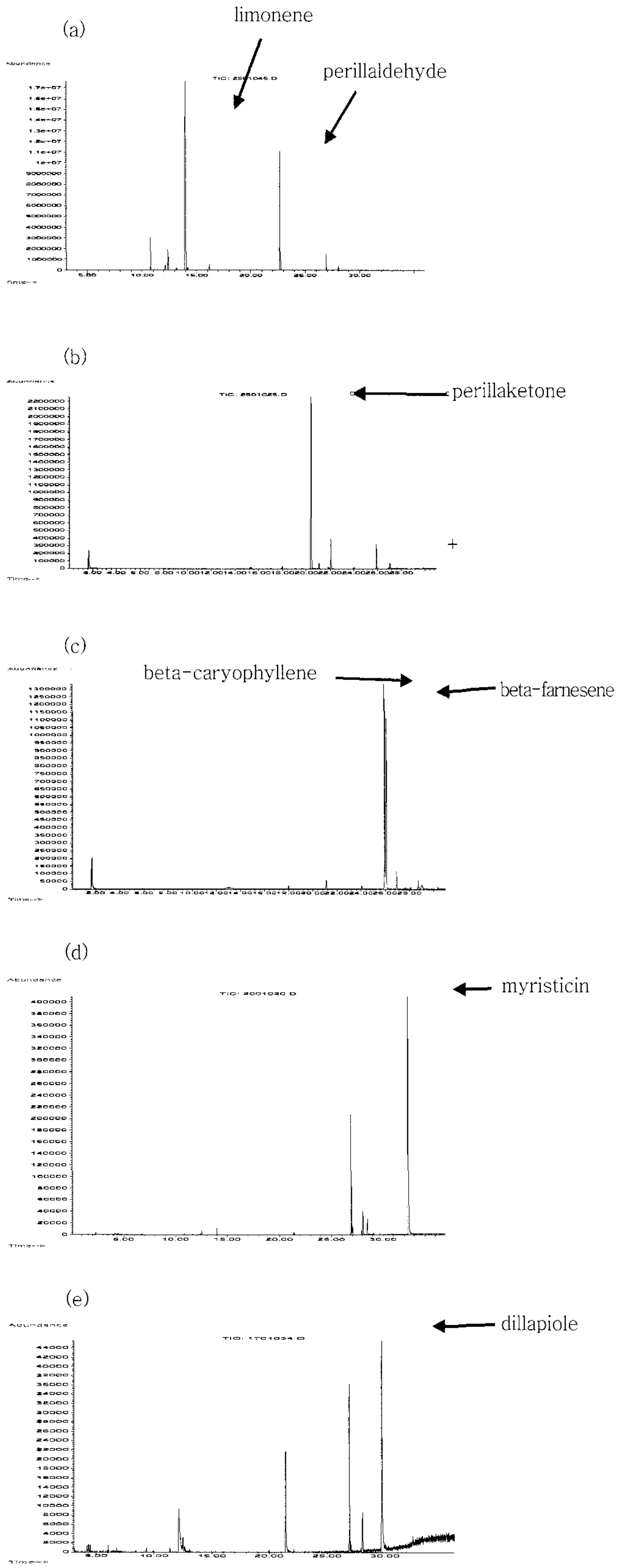

Fig. 1. Total ion chromatograph of GC/MS spectrum of headspace analysis from leaf of jaso.

patterns obtained by GC-MS analysis. Major component of (d) and (e) in Fig. 1 were myristicin and dillapiole, respectively, which belong to phenylpropenes. Therefore, these two patterns could be classified into one type ; PP type (phenylpropene type). The major components showed in Fig. 1-(c) were beta-caryophyllene and beta-farnesene which belong to sesquiterpenes and this type might be classified into ST type (sesquiterpene type). As a results, the chemotypes based on the contents of the major volatile component of Jaso grown in Korea could be classified into four types.

The contents of major volatile component in each chemotype were shown in Table 3. PA type occupied 41.9% out of total sample analyzed and 57.9% of it was limonene and 19.6% was perillaldehyde. This type was detected in all Jaso varieties (wild perilla, ruffle-leaved purple perilla, upper-green purple perilla, purple perilla) collected in this study. PA type may provide the possibility of selection as a vegetable perilla with special aroma and red leaf instead of vegetable perilla with tough and strong aroma at present consumed. PK type contained 89.8%, perillaketone which was identified with 38.8% out of total sample analyzed. Perillaketone has been reported as a major volatile component in Deulkkae (*P. frutescens* Brit. var. *japonica* Hara) grown in Korea (Jeong *et al.*, 1998; Kim *et al.*, 1999; Lee *et al.*, 1999; Song *et al.*, 1998). Therefore, PK type is expected to be cultivated as leaf vegetable which has the same fragrance of Deulkkae but differ from

Table 3. The major volatile component of chemotype of *P. frutescens*.

Major component	GC area %			
	Chemotype			
	PA type	PK type	ST type	PP type
PA	77.54+5.99	0	4.92+10.10	8.37+ 9.09
PK	0.15+0.50	89.84+5.95	0	4.47+ 6.99
ST	9.07+5.47	5.02+3.04	82.37+ 9.56	37.83+ 8.54
PP	0.46+2.75	0.01+0.05	1.42+ 1.15	40.32+11.29

PA : limonene (57.7%)+perillaldehyde (19.8%).
PK : perillaketone.
ST : beta-caryophyllene(54.5%+beta-farnesene (27.9%).
PP : myristicin (13.6%)+dillapiole (26.7%).

Deulkkae in color and shape of leaf. ST type contained 82.4% sesquiterpenes (54.5% beta-caryophyllene and 27.9% beta-farnesene), which was identified with 7.8% out of total sample analyzed. This type, not reported in Japan, was classified newly in this study. Sesquiterpenes are generally less volatile and have less direct organoleptic properties than monoterpenes. ST type could be used to develop cultivar for consumer who do not like Deulkkae as leaf vegetable with strong and tough aroma. PP type was containing 40.3% phenylpropenes (13.6% myristicin and 26.7% dillapiole) and 37.8% sesquiterpenes, which was 11.6% out of total sample analyzed.

ACKNOWLEDGEMENT

This research was supported by a grant (code # CG3213) from Crop Functional Genomics Center of the 21st Century Frontier Research Program funded by the Ministry of Science and Technology of Republic of Korea.

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