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Full Length Article

Characterization of Aroma-Active Compounds from Sweet Osmanthus (*Osmanthus fragrans*) by SDE and SPME Coupled with GC-MS and GC-Olfactometry

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Abstract

Aroma volatiles from ripened and fresh flowers of sweet osmanthus (*Osmanthus fragrans* Lour.) from Asiaticus group were extracted by simultaneous distillation-extraction (SDE) and solid-phase microextraction (SPME). They were then identified and characterized using gas chromatography-mass spectrometry (GC-MS) and GC-olfactometry (GC-O). A total of 84 volatiles were detected by GC-MS analysis. Among them, terpenoids, ketones and esters were predominant in SPME extracts, while aromatics and alcohols were abundant in SDE extracts. With the two methods, 13 and 15 aroma-active compounds were characterized in ripened and fresh flowers, respectively. The aroma-active compounds with similar sensory descriptors were grouped into the same category and the modified frequency (MF) values of each category were summed up. The results showed that, fresh flowers seemed to show more herbal (147 to 83%), citrus/green/fresh (175 to 126%) and earthy (60 to 0%) odor. On the other hand, while ripened flowers seemed to show more floral/rose (243 to 196%) and paint/tar (118 to 0%) odor and both of them presented the violet/woody/fruity odor. It was indicated that the combination of multiple extraction methods, GC-MS and GC-O analysis can enhance the accuracy of identification and provide a reference for the further study on flavor of flower products.

Keywords: Sweet osmanthus; Volatiles; Solid-phase microextraction; Simultaneous distillation-extraction

Introduction

Sweet osmanthus (*Osmanthus fragrans* Lour.), belonging to the family Oleaceae, is a well-known ornamental germplasm native to East Asia. Based on phenotypes such as the leaf shape, flower color, aroma, season and frequency of flower blooming, they are divided into four cultivar groups, including Albus group, Luteus group and Aurantiacus group which are blooming only in autumn and Asiaticus group which blooms during most part of the year (Zang and Xing, 2004; Yang *et al.*, 2018). Owing to the unique scent and biological properties, commercial extracts from fresh and ripened flowers of sweet osmanthus are in high demand for use in the production of expensive cosmetics, functional food additives and traditional Chinese medicines (Wu *et al.*, 2009; Zhou *et al.*, 2018).

Gas chromatography-mass spectrometry (GC-MS) is useful for aroma profile analyses that have been applied in sweet osmanthus to improve food qualities. It showed that the volatiles varied likely depending on extraction methods and the cultivars (Wu *et al.*, 1997; Hu *et al.*, 2009; Cai *et al.*, 2014; Zou *et al.*, 2017). Solid-phase microextraction (SPME), free of solvent, is the fastest and most sensitive technique to obtain aroma characteristics of fresh food (Kaseleht *et al.*, 2011). Simultaneous distillation extraction (SDE), which combines the advantage of liquid-liquid and steam distillation extractions, is considered effective to extract volatiles for ripened food (Chaintreau, 2001). It was showed that γ-decalactone was the common composition detected by SDE (Hu *et al.*, 2012), while, *trans*-geraniol, β-ionone and linalool were detected as the major compounds analyzed by SPME (Xin *et al.*, 2013). Most of these studies were focused in cultivars blooming in autumn. However, our previous study reported that the short flowering period of autumn blooming cultivars has limited their economic value (Zou *et al.*, 2014). Thus the characterization of aroma compounds of Asiaticus group cultivars, which bloom in almost four seasons, is necessary.

Furthermore, although the information obtained by GC-MS is useful for understanding qualitative profiles, it was incomplete to provide an accurate indication of aroma as it lacks odor perceptions (Cullere *et al.*, 2011). GC-olfactometry (GC-O) is an effective method to determine aroma-active compounds, which actually contributed to aroma (Lee, 2003). Thus, volatiles and aroma-active compounds of flowers in *O. fragrans* 'Siji Gui', a widely

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planted cultivar from Asiaticus group, were extracted by SPME and SDE respectively and then analyzed by GC-MS and GC-O in the present study, with the purpose to improve the application of ever-blooming cultivars of sweet osmanthus and to improve food qualities on flavor of flower products by a more accurate and effective method.

Materials and Methods

Plant Materials

Flowers of *O. fragrans* 'Siji Gui' were harvested at the full flowering stage from mature tree grown in the nursery of Huazhong Agricultural University (Wuhan, China) (114°22'W, 30°29'N). The essential oil extracted by SDE and fresh flowers resolved by SPME were used to analyze the volatiles and aroma-active compounds.

SDE Extraction

Essential oil samples were isolated from fresh material (10 g florets plus 250 mL of distilled water) by SDE for 1 h, using a modified Likens–Nickerson apparatus (Seidel and Lindner, 1993) with absolute ether (25 mL). The essential oils obtained by SDE were cooled to -20°C to separate the frozen water from the organic phase by decantation. The extracts were dried over anhydrous sodium sulphate and concentrated to a final volume 1.0 mL using a gentle stream of nitrogen.

SPME Extraction

The extraction process was performed by the method described in Cai *et al.* (2014). A total of 2.0 g fresh florets were added into a 20 mL screw cap glass vial with a Teflon silica septum placed at room temperature (25±2°C) for 30 min of equilibration.

GC-MS Analysis

The GC-MS system and conditions were the same as described in Cai *et al.* (2014). The system was comprised of a TRACE GC Ultra GC coupled to a DSQ II mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA).

GC-O Analysis

The GC-O equipment and time-intensity method were the same as described by Cai *et al.* (2014). The analysis was performed on a HP 6890 GC coupled with an Agilent 5975 Network Mass Spectrometer (Agilent Technologies, Palo Alto, CA, USA) and equipped with a sniffing port (ODP2, Gerstel, Inc., Baltimore, MD, USA). For measuring the intensity, five evaluation scores were used as described by Cai *et al.* (2014). The data processed were a mixture of the intensity and the frequency of detection of an odorant, labeled as 'modified frequency' (MF): MF (%) = [F (%)×I (%)]½. F (%) is the detection frequency of an aromatic attribute expressed as a percentage of total number of judges and I (%) is the average intensity expressed as percentage of the maximum intensity.

Components Identification

Identifications were based on matching the totality of data from MS fragmentation patterns in the National Institute of Standards and Technology (NIST 08), retention index values calculated using a homologous series of n-alkanes standards (C_8-C_{20}, Fluck) on HP-5 columns, sensory descriptors as well as with odor qualities from Flavornet (www.flavornet.org) of target substances with those of authentic standards.

Statistical Analysis

Three biological replications of each extraction were performed. The differentiation of volatiles between essential and fresh flower of sweet osmanthus was performed with the Student t test at $p = 0.05$, using SAS 8.0 software.

Results

GC-MS Analysis of Volatile Compounds

SDE and SPME were used to examine volatiles of ripped and fresh flowers of sweet osmanthus. With the two methods, 54 and 60 volatiles were characterized in SDE and SPME method, respectively. The volatiles were assigned to different chemical families. In SDE extracts, there were 14 terpenoids, 11 ketones, 12 hydrocarbons aromatics, 4 esters, 4 alcohols, 7 aldehydes and 3 alcanes. On the other hand, volatile compounds in fresh flower included 25 terpenoids, 8 ketones, 9 hydrocarbons aromatics, 11 esters and 6 alcohols (Table 1).

Terpenoids was the most abundant volatile class in fresh flower. Linalool (19.21%) and *(E)*-*β*-ocimene (12.28%) were detected as major terpenoids in fresh flowers. However, 2, 4-dimethyl-1-heptene (2.36%), *(Z)*-linalool oxide (2.17%) and *(Z)*-geraniol (2.66%) showed higher proportions in extracts of ripped flowers. Ketones and esters have been described to be most important to sweet osmanthus flavor. Among the ketones, *(E)*-*β*-ionone and dihydro-*β*-ionone were most predominant volatiles occurring in higher proportions in fresh flower of sweet osmanthus. 3, 7-dimethyl-1, 6-octadien-3-ol formate, which was the most major ester (8.7%) in fresh flower, have not been detected in ripped flowers. Furthermore, aldehydes and alcanes were only detected in extracts of ripped flowers.

GC-O Analysis of Aroma-active Compounds

As shown in Table 2, only 18 compounds (13 by SDE and 15 by SPME) were detected to contribute the aroma of sweet osmanthus flowers. Among them, linalool's derivates and *(Z)*-geraniol had higher MF values in extracts of ripped flowers, while MF values of ocimene, linalool and ketones was higher in fresh flowers.

Table 1: Volatile compounds in essential oil and fresh flower of sweet osmanthus

[1)]RI	[2)]RI	Name	Ripped flower	Fresh flower	[7)]ID method
			[3)]RC ± [4)]SD	[3)]RC ± [4)]SD	
		Alcohols			
732		1,2-benzenediol, 4-[2-(methylamino)ethyl]-	[6)]U	0.45±0.13	M
869	869	*(E)*-3-hexen-1-ol	0.37±0.05[5)]*	0.11±0.07	M, R, C
1299		Phenol, 2-cyclohexyl-4-methyl-	0.02±0.015	0.02±0.01	M, C
1332	1328	*(Z)*-edulan	U	0.72±0.09	M, R
1391		1,4,4,7a-tetramethyl-2,4,5,6,7,7a-hexahydro-1H-indene-1,7-diol	U	0.29±0.05	M
1427	1428	*β*-ionol	2.32±0.28	U	M, R
1448		4-(2,6,6-trimethyl-cyclohex-1-enyl)-butan-2-ol	1.87±0.12*	0.05±0.01	M
		Aldehydes			
725	725	Acetal	1.53±0.13	U	M, R, C
902	902	Heptanal	0.64±0.22	U	M, R, C
1007	1007	Octanal	0.65±0.08	U	M, R, C
1108	1108	Nonanal	12.10±1.22	U	M, R, C
1206	1206	Decanal	0.25±0.08	U	M, R, C
1209		2-methyl-4-octenal	0.85±0.08	U	M
1611	1611	Tetradecanal	0.40±0.05	U	M, R, C
		Aromatics			
782	782	Toluene	1.63±0.07	U	M, R, C
859	859	Ethylbenzene	1.51±0.14	U	M, R, C
867	867	*p*-xylene	0.89±0.07	U	M, R, C
888	888	*o*-xylene	0.71±0.24	U	M, R, C
917	917	2-amino-5-methylbenzoic acid	0.29±0.04*	0.12±0.05	M, R, C
1039	1039	Benzeneacetaldehyde	0.42±0.09	U	M, R, C
1169	1169	1,2,3,4-tetramethylbenzene	0.74±0.15	U	M, R, C
1192	1192	Naphthalene	0.73±0.08*	0.01±0.01	M, R, C
1291	1291	2-methylnaphthalene	1.35±0.12*	0.03±0.02	M, R, C
1297	1297	Theaspirane	0.83±0.12*	0.03±0.01	M, R, C
1307	1307	1-methylnaphthalene	0.42±0.09*	0.02±0.01	M, R, C
1354	1354	1,2-dihydro-1,5,8-trimethylnaphthalene	U	0.28±0.04	M, R
1358	1355	1,1,4,5-tetramethylindan	U	0.18±0.05	M, R
1372		Benzene, 1-ethyl-3,5-diisopropyl-	U	0.02±0.01	M
1517	1514	Butylated hydroxytoluene	0.68±0.03*	0.01±0.01	M, R, C
		Alcanes			
960	960	4-methylnonane	0.25±0.07	U	M, R, C
988	989[m1]	2,2,4,6,6-pentamethylheptane	0.07±0.02	U	M, R, C
1893	1893	Heptadecane,2,6,10,15-tetramethyl-	0.70±0.11	U	M, R
		Esters			
1012	1008	*(Z)*-3-hexenol acetate	U	1.17±0.10	M, R, C
1098		*(Z)*-pent-2-enyl butyrate	U	0.02±0.01	M
1150	1145	*(Z)*-3-hexenyl isobutanoate	U	0.01±0.00	M, R, C
1191	1188	*(Z)*-3-hexenyl butanoate	U	0.35±0.07	M, R, C
1197	1193	Hexyl butanoate	U	0.02±0.02	M, R, C
1237	1237	*(Z)*-3-hexenyl-2-methylbutyrate	0.65±0.08*	0.02±0.01	M, R, C
1243	1240	*(Z)*-3-hexenyl isovalerate	U	0.20±0.08	M, R, C
1260	1256	3,7-dimethyl-1,6-octadien-3-ol formate	U	8.70±0.36	M, R, C
1386	1382	*(Z)*-3-hexenyl hexanoate	U	0.02±0.01	M, R, C
1463		Spiro[4.5]decane-1,6-dione	0.06±0.02	0.05±0.01	M
1471	1475	*γ*-decalactone	12.07±1.77*	4.73±0.38	M, R, C
1873	1873	Isobutyl phthalate	0.22±0.11	U	M, R, C
		Ketones			
795	798	Isopropylideneacetone	3.80±0.30	U	M, R, C
816	819	3-hexen-2-one	U	0.02±0.02	M, R
993	993	Sulcatone	U	0.07±0.04	M, R, C
1186	1186	Cryptone	0.94±0.09	U	M, R
1206	1206	Berbenone	0.25±0.08	U	M, R
1402	1404	*(Z)*-jasmone	1.11±0.14	0.92±0.07	M, R, C
1431	1430	*α*-ionone	1.23±0.19	3.59±0.38*	M, R, C
1442	1443	Dihydro-*β*-ionone	5.22±0.28	6.28±0.29*	M, R, C
1459	1458	Geranyl acetone	0.12±0.03*	0.01±0.01	M, R, C
1490	1491	*(E)*-*β*-ionone	11.56±0.92	26.28±1.19*	M, R, C
1500	1496	*β*-ionone	0.31±0.19*	0.05±0.03	M, R, C
1784		Diphenylcyclopropenone	0.39±0.11	U	M, C
1850	1845[m2]	Perhydrofarnesyl acetone	0.74±0.08	U	M, R
		Terpenoid			
835	842	2,4-dimethyl-1-heptene	2.36±0.12	U	M, R
998	995	*β*-myrcene	U	2.35±0.16	M, R, C
1005	1005	*L*-*β*-pinene	U	0.06±0.02	M, R, C
1018	1018	3-carene	U	0.03±0.02	M, R, C
1030	1030	*d*-limonene	U	1.39±0.14	M, R, C
1036	1036	Limonene	U	0.02±0.02	M, R, C
1042	1042	*(Z)*-*β*-ocimene	0.33±0.06	0.76±0.13*	M, R, C
1051	1051	*(E)*-*β*-ocimene	1.07±0.05	12.28±0.68*	M, R, C
1061	1060	*γ*-terpinen	U	0.13±0.05	M, R, C
1075	1075	*(Z)*-linalool oxide	2.17±0.19*	0.64±0.13	M, R, C
1085	1088	Isoterpinolene	U	0.02±0.01	M, R
1091	1091	*(E)*-linalool oxide	1.99±0.17*	0.82±0.09	M, R, C

Table 1: Continued

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1103	1103	Linalool	0.86±0.12	19.21±0.32*	M, R, C
1106	1109	Hotrienol	U	0.02±0.02	M, R
1110	1119	6-ethenyldihydro-2,2,6-trimethyl-2H-pyran-3(4H)-one	U	0.01±0.00	M, R
1121		2-ethenyl-1,1-dimethyl-3- methylene-cyclohexane	0.03±0.02	0.08±0.04	M
1126	1130	*(E)*-2,6-dimethyl-1,3,5,7-octatetraene	0.02±0.01	0.03±0.02	M, R
1134	1132	Allo-ocimene	0.02±0.02	1.76±0.20*	M, R, C
1146	1146	Neo-allo-ocimene	U	0.67±0.09	M, R
1172	1173	Epoxylinalol	0.73±0.04*	0.12±0.04	M, R
1178	1174	*(Z)*-linalool oxide (pyranoid)	0.68±0.15*	0.14±0.03	M, R
1233	1233	*(Z)*-geraniol	2.66±0.10*	0.03±0.03	M, R, C
1267	1251	Megastigma-4,6*(Z)*,8*(Z)*-triene	U	0.03±0.02	M, R
1276	1276	Citral	0.37±0.08	0.43±0.07	M, R, C
1344	1354	Megastigma-4,6*(E)*,8*(Z)*-triene	U	0.05±0.02	M, R
1364	1360	Megastigma-4,6*(E)*,8*(E)*-triene	0.21±0.04	0.21±0.03	M, R
		Others			
753		Acetic acid, hydroxyl [(1-oxo-2-propenyl)amino]-	U	0.14±0.05	M

[1)] Retention index on HP-5 column calculated in present study
[2)] Retention index obtained from the NIST Chemistry Web Book (http://webbook.nist.gov/chemistry)
[3)] RC: relative content calculated on the basis of chromatographic peak areas
[4)] SD: standard deviation (3 replicates)
[5)] Relative contents labeled asterisk (*) indicates significant differences between ripped and fresh flowers of sweet osmanthus performed with the Student t test ($p < 0.05$)
[6)] Not detected
[7)] ID method: M = Comparison of their mass spectrum to reference databases, R = comparison of retention index, C= comparison with reference compounds

Table 2: Aroma-active compounds in essential oil and fresh flower of sweet osmanthus

Chemical families	Nos.	RI	Name	Odor descriptors	MF (%)		[2)]ID method
					SDE	SPME	
Aldehydes	1	1108	Nonanal	Fat, citrus, green	93	[1)]U	R, O, M, C
Aromatics	2	782	Toluene	Paint	58	U	R, O, M, C
	3	1354	1,2-dihydro-1,5,8-trimethylnaphthalene	Earthy	U	60	R, O, M
Esters	4	1191	*(Z)*-3-hexenyl butanoate	Green, Banana	U	28	R, O, M, C
Ketones	5	1431	*α*-ionone	Woody, violet, fruity	73	80	R, O, M, C
	6	1442	Dihydro-*β*-ionone	Fruit, sweet, wood	80	87	R, O, M, C
	7	1489	*(E)*-*β*-ionone	Violets, woody	93	99	R, O, M, C
Terpenoid	8	1029	*d*-limonene	Citrus, minty	U	52	R, O, M, C
	9	1047	*(Z)*-*β*-ocimene	Herbal, floral	33	65	R, O, M, C
	10	1051	*(E)*-*β*-ocimene	Herbal	50	82	R, O, M, C
	11	1074	*(Z)*-linalool oxide	Flower	65	33	R, O, M, C
	12	1091	*(E)*-linalool oxide	Flower, green	60	45	R, O, M, C
	13	1103	Linalool	Floral, lavender	60	96	R, O, M, C
	14	1133	Allo-ocimene	Fresh	U	28	R, O, M, C
	15	1145	Neo-allo-ocimene	Fresh, sweet	U	45	R, O, M
	16	1178	*(Z)*-linalool oxide (pyranoid)	Citrus, green	33	22	R, O, M
	17	1233	*(Z)*-geraniol	rose, geranium	58	22	R, O, M, C
	18	1291	2-methylnaphthalene	tar	60	U	R, O, M, C

[1)]Not detected
[2)]ID method: M = Comparison of their mass spectrum to reference databases, R = comparison of retention index, C= comparison with reference compounds. O = odor described by panelists, compared to Flavornet (www.flavornet.org)

Other compounds contributed to the overall aroma of sweet osmanthus were toluene (58%), 2-methylnaphthalene (60%) and nonanal (93%) and they appeared only in extracts of ripped flowers. In addition, *(Z)*-3-hexenyl butanoate (28%), 1, 2-dihydro -1,5,8-trimethylnaphthalene (60%) accompanied with other monoterpenes, *d*-limonene (52%), allo-ocimene (28%) and neo-allo-ocimene (45%) were the aroma-active compounds with lower MF values that were only detected in fresh flower.

To analyze the aroma profiles, aroma-active compounds were divided into different groups based on the similarity of their aroma descriptors (Fig. 1). The ketones, including *α*-ionone, dihydro-*β*-ionone and *(E)*-*β*-ionone, that have been confirmed to contribute to fruit, woody and violets flavor, have the highest MF values than other groups in both ripped flowers (246%) and fresh flowers (266%). Fresh flowers seemed to show more herbal (147 to 87%) and citrus/green/fresh (175 to 126%) odor because of the significantly higher MF values of ocimenec, *(Z)*-linalool oxide (pyranoid), d-limonene and *(Z)*-3-hexenyl butanoate. On the other hand, ripped flowers have showed more floral/rose (243 to 196%) odor because of a significantly higher MF values of *(Z)*-*β*-ocimene and *(E)*-*β*-*o*cimene. Additionally, paint/tar (118%) odor could only be detected in SDE extracts and earthy (60%) odor could only be detected in SPME extracts.

Discussion

By SDE method, 54 volatile compounds in ripped flowers of sweet osmanthus were detected and the most abundant components were nonanal, *γ*-decalactone and *(E)*-*β*-ionone (Table 1). It was reported that, with hydrodistillation method, 48 components were identified, and *α*-methyl-α-[4-methyl-3-pentenyl] oxiranemethanol and epoxylinalool were the major constituents (Hu *et al.*, 2009).

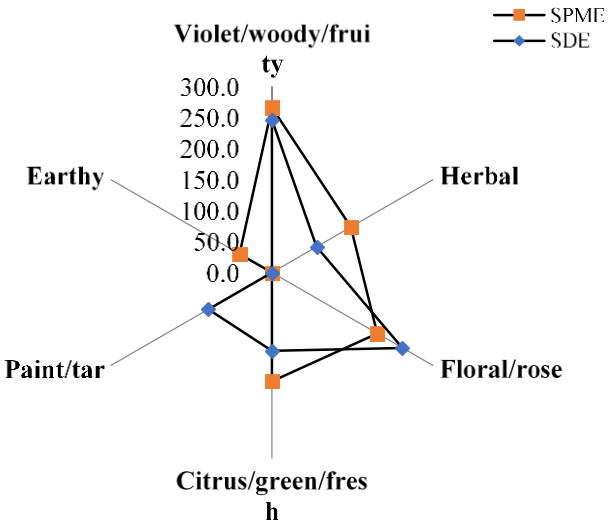

Fig. 1: Graph of mean sensory ratings MF (%) of different aroma-active compounds between essential oil (SDE) and fresh flower (SPME) of sweet osmanthus

With supercritical fluid extraction (SFE) method, 57 volatile compounds were reported, with methyl eicosatlrienoate, hexadecanoic acid and 4-hydroxy-*β*-ionone as the primary constituents (Wu *et al.*, 1997). With SPME method, 60 volatile compounds in fresh flowers were obtained in the present study and *(E)*-*β*-ionone, linalool and *(E)*-*β*-ocimene were predominant constituents (Table 1). Thus, it can be seen that the variations in volatiles likely depended on extraction methods.

Terpenoids was the most abundant volatile class in fresh flower. Most of these terpenoids compounds in sweet osmanthus were monoterpenes and its oxides. It suggested that SPME showed preference in determine these compounds because of their high volatility (Prakash *et al.*, 2012). Toluene, ethylbenzene, *p*-xylene and *o*-xylene were only detected in SDE extracts. They were alkylated benzenes, and widely occurred in industrial solvents, paints and chemical products (Vichi *et al.*, 2005). Thus, these compounds possibly came from the interference of organic solvent in SDE. In SDE results, nonanal showed a higher percent than other aldehydes, which are most likely enzymatic degradation products of unsaturated fatty acids such as oleic acid, linoleic acid and linolenic acid (Lin and Rouseff, 2001).

Not all the volatile compounds found in greatest concentration contributed to the odors of sweet osmanthus. For example, *γ*-decalactone, one of the main volatile in extracts from both ripped and fresh flower, was not perceived by panelists as an aroma-active compound. *β*-myrcene, which had been reported as a common monoterpene in other species (Liu *et al.*, 2012; Si *et al.*, 2012), had no aroma contribution in sweet osmanthus. Isopropylideneacetone was the important volatile in essential oil, but did not present typical odor of sweet osmanthus. These results implied that the predominant volatile compounds were not necessarily the same as the aroma-active compounds.

As two abundant volatile classes in both ripped and fresh flowers, terpenoids and ketones were also the most important aroma-active compounds in sweet osmanthus. Linalool and its derivates, with high percent by GC-MS analysis, were also important aroma active compounds presenting flower/lavender notes (Kilic *et al.*, 2004). *(Z)*-geraniol, which was generally considered an important contributor to rose and grapefruit oil flavor (Lin and Rouseff, 2001; Jirovetz *et al.*, 2005), has been described to impart rose and geranium odor in sweet osmanthus. *(Z)*-*β*-ocimene and *(E)*-*β*-ocimene, which released the herbal odor in flowers of sweet osmanthus, have been reported as aroma-active compounds presented herbal and grassy odor in leaves and stems (Zheng *et al.*, 2004). The odors of *(E)*-*β*-ionone, *α*-ionone and dihydro-*β*-ionone, described as woody, fruity, and violet flavor in sweet osmanthus, were also identified in fruits (Miyazaki *et al.*, 2012). Linalool and ionones have been considered as important flavor and fragrance compositions with high consumption in the world (Schwab *et al.*, 2008). Their high odor intensities in flowers of 'Siji Gui' indicated its potential economic value.

Conclusion

A total of 84 volatiles were detected by GC-MS analysis, but only 18 compounds of them contributed the aroma to sweet osmanthus. Fresh flowers seemed to show more herbal, citrus/green/fresh and earthy odor; while ripened flowers seemed to show more floral/rose and paint/tar odor. It is suggested that aroma-active compounds detected by GC-O analysis were likely the crucial and characteristic aromatic compounds determining the special flavor of ripened and flesh flowers of sweet osmanthus. This study provided a reference for the further study on flavor of flower products.

Acknowledgments

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