Kor. J. Hort. Sci. Technol. 29(4):382-387, 2011

A Multivariate Statistical Approach to Comparison of Essential Oil Composition from Three *Mentha* Species

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Abstract. The chemical composition of essential oils obtained from aerial parts in spearmint, apple mint and chocolate mint, was investigated by gas chromatography/mass spectrometry analyses. (-)-Carvone (33.0%) was quantitatively major compound in spearmint, followed by R-(+)-limonene (11.7%) and β-phellandrene (9.7%); (-)-carvone (37.4%) and germacrene D (11.9%) in apple mint; and (-)-menthol (34.3%), *p*-menthone (18.4%) and menthofuran (9.8%) in chocolate mint. Hierarchical cluster analysis and principle components analysis showed the clear difference in chemical composition of the three mint oils.

***Additional key words*:** apple mint, chocolate mint, hierarchical cluster analysis, principle component analysis, spearmint

Introduction

Mints (*Mentha* spp.), an aromatic perennials in the family Lamiaceae, contain about 40 species including hybrids and are distributed across Europe, Africa, Asia, Australia and North America (Park, 2003). They are one of the most important aromatic plants due to their essential oil with the distinctive flavor and strong biological activity. The characteristics of mint essential oil have led to be widely used as flavorings in food, mouth fresheners, cosmetics and perfumes. The mint oil is also used as pharmaceuticals such as astringents, tonics, mild laxatives and antibacterials (Agnihotri et al., 2005).

A quantitative and qualitative variation of odor-active compounds in essential oils contributes to different flavor type of aromatic plants (Mirshekari, 2010; Mirshekari et al., 2009; Sangwan et al., 2001). For example, trans-anethole, estragole, fenchone and 1-octen-3-ol contributes to overall aroma of fennel seed with anise-like aroma (Diaz-Maroto et al., 2005). However, hops with a bitter and tangy flavor are composed of trans-4,5-epoxy-(*E*)-2-decenal, linalool, and myrcene as key compounds contributing to the aroma of hops (Steinhaus et al., 2000). A variety of mint species have various flavor types: Spearmint oil is less pungent than peppermint oil; a strongly aromatic and bitter flavor in peppermint oil; a fruity and spearmint flavor in apple mint oil; a chocolate-peppermint scent in chocolate mint oil; a sweet and basil-mint aroma in field mint oil (Bown, 2001). Their mint oils have been reported to be composed of different chemical composition (Agnihotri et al., 2005; Barton et al., 1992; Dwivedi et al., 2004; Hussain et al., 2010). In addition to flavor type, the different chemical composition in essential oil also affects a variety of biological activities against microorganisms (Vukovic et al., 2009). Essential oils from mints were reported to have important antimicrobial activities (Hussain et al., 2010; Mahboubi et al., 2008).

Therefore, mint species can have different flavor and a variety of biological activities is based on the chemical composition of the essential oils. In this study, chemical compositions of essential oil from aerial parts in spearmint, apple mint and chocolate mint with distinctly different flavor types were analyzed using GC-MS and compared through multivariate analyses (i.e., hierarchical cluster analysis and principal component analysis) to evaluate the variation in the chemical composition and characterize the most important flavor compounds which contributes to the difference among samples.

Materials and Methods

Plant Materials

The fresh leaves of spearmint (*Mentha spicata*), apple mint (*M. suaveolens*), and chocolate mint (*M.* × *piperita* 'Chocolate') were harvested from the research farm (37° N/127° E) at Korea University, Seoul, Korea in March 2008. All plant samples were washed and air-dried before isolating the essential oil of each sample.

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※ Received 21 June 2011; Accepted 14 July 2011.

Essential Oil Isolation

The fresh leaves (300 g) and water (4 L) in a 10-L round-bottom flask were subjected to simultaneous distillation extraction (SDE) for 3 hours using a modified Likens-Nickerson apparatus. Diethyl ether (purity 99.9%) was used as a solvent. After isolating essential oil from each sample, water trace in essential oil was eliminated using anhydrous sodium and the solvent was evaporated with a rotary evaporator (EYERA, Tokyo, Japan) under reduced pressure. The extracted essential oils were stored at 4℃ prior to analysis.

GC–MS Analysis

The essential oil samples were analyzed by gas chromatography/mass spectrometer (GC/MS, Model 7890N/5973, Agilent, Palo Alto, CA). Separation of the essential oil compounds was performed using a GC/MS equipped with a 30 m length, 0.25 mm i.d., 0.25 μm film thickness (5% phenyl methyl silicone), fused silica capillary column (DP-5MS, Agilent, Palo Alto, CA). Injection port temperature was 200℃ with a splitless mode. Helium was used as the carrier gas at a flow rate of 3 mL/minute. The column temperature was held at 50℃ for 5 min and then increased at 10℃/minute to 250℃, held for 5 min. MS conditions were: ion source 230℃; electron energy 70 eV; multiplier voltage 1258 V; GC/MS interface zone 280℃; and a scan range of 50 to 550 mass units.

The essential oil compounds were tentatively identified based on comparison of their mass spectra and relative abundances with NIST 07 and those reported in the literature (Adams, 2007). Normal alkane retention indexes (RI) were determined using a nonpolar DB-5MS column and a series of *n*-hydrocarbons (C7-C22) and comparing with those listed at http://webbook.nist.gov/chemistry/. The results were expressed as the average of three replicates.

Statistical Analysis

Hierarchical cluster analysis (HCA) and principal component analysis (PCA) were carried out by using the SAS system for Windows v8 (SAS Institute, Cary, NC) to assess the differences in chemical composition of essential oil among mint samples. HCA used Ward's minimum variance method with a Euclidean distance and PCA used the covariance matrix obtained from data matrix (Khattree and Naik, 2000).

Results and Discussion

Chemical Composition of the Essential Oils

The extraction yield (g·100 g^{-1} of fresh weight) of essential oil from the aerial parts of spearmint, apple mint and chocolate mint was 0.7, 0.4 and 1.0% (w/w), respectively. Their chemical composition qualitatively and quantitatively varied such as the extraction yields. Forty-eight, fifty-three and forty-seven compounds in spearmint, apple mint and chocolate mint were tentatively identified (Table 1). Sixteen compounds were found in all species, while 8 compounds were detected

Table 1. Chemical composition (%) of the essential oils of three *Mentha* Species by GC-MS Analysis.

RI[z]	Compound	Relative composition (%)		
		Spearmint	Apple mint	Chocolate mint
	Monoterpenoids			
930	α-Pinene	0.3 ± 0.0[y]	0.9 ± 0.0	0.1 ± 0.0
931	α-Thujene	2.6 ± 0.2	ND	ND
970	Sabinene	3.3 ± 0.1	ND	ND
980	β-Pinene	0.4 ± 0.0	0.8 ± 0.0	2.7 ± 0.9
986	*p*-Menth-3-ene	ND	ND	5.8 ± 2.0
990	Myrcene	5.6 ± 0.1	5.4 ± 0.3	ND
1013	α-Terpinene	ND	ND	4.5 ± 1.9
1021	*m*-Cymene	ND	ND	2.7 ± 1.1
1032	β-Phellandrene	9.7 ± 0.3	1.9 ± 0.1	ND
1035	Limonene	11.7 ± 0.3	1.9 ± 0.1	0.8 ± 0.3
1033	Eucalyptol	0.5 ± 0.5	0.8 ± 0.1	1.9 ± 0.8
1041	(*E*)-Ocimene	2.1 ± 1.0	1.7 ± 0.0	ND
1045	(*Z*)-Ocimene	1.0 ± 0.1	0.9 ± 0.1	ND
1060	γ-Terpinene	0.6 ± 0.1	1.3 ± 0.0	0.5 ± 0.1
1068	Sabinene hydrate	0.4 ± 0.1	0.8 ± 0.1	0.2 ± 0.1
1080	Terpinolene	0.2 ± 0.0	0.4 ± 0.0	0.3 ± 0.1

Table 1. (Continued)

RI[z]	Compound	Relative composition (%)		
		Spearmint	Apple mint	Chocolate mint
1093	β-Linalool	0.1 ± 0.0	1.1 ± 0.1	ND
1138	(+)-(E)-Limonene oxide	0.1 ± 0.0	ND	ND
1154	*p*-Menthone	0.1 ± 0.0	0.1 ± 0.0	18.4 ± 3.6
1162	Borneol	1.3 ± 1.0	ND	ND
1163	Menthofuran	ND	ND	9.8 ± 9.0
1174	(±)-Menthol	ND	ND	34.3 ± 6.9
1182	(-)-Terpinen-4-ol	0.3 ± 0.1	0.5 ± 0.1	ND
1183	*p*-Cymen-8-ol	ND	ND	1.0 ± 0.6
1193	Dihydrocarvone	0.8 ± 0.1	0.2 ± 0.0	ND
1192	α-Terpineol	ND	ND	0.1 ± 0.1
1194	(-)-Myrtenol	ND	ND	1.7 ± 1.4
1246	Carveol	ND	0.1 ± 0.0	4.4 ± 3.1
1252	Piperitone	ND	ND	2.7 ± 0.6
1262	(-)-Carvone	33.0 ± 1.9	37.4 ± 0.4	ND
1263	cis-Carvone oxide	ND	0.6 ± 0.6	ND
1290	Thymol	ND	ND	0.4 ± 0.2
1344	Piperitenone	0.9 ± 0.2	0.1 ± 0.2	ND
1361	Eugenol	ND	ND	0.4 ± 0.0
1396	Isoeugenol	ND	0.6 ± 0.0	Tr
1404	Methyl eugenol	ND	0.5 ± 0.0	ND
	Sesquiterpenoids			
1350	α-Cubebene	ND	0.1 ± 0.0	ND
1371	α-Ylangene	ND	ND	0.3 ± 0.1
1372	(-)-α-Copaene	0.4 ± 0.1	0.5 ± 0.0	ND
1379	β-Bourbonene	1.7 ± 0.5	2.5 ± 0.1	ND
1419	Caryophyllene	0.4 ± 0.2	0.1 ± 0.0	0.2 ± 0.1
1447	(+)-Aromadendrene	ND	ND	0.3 ± 0.1
1459	β-Farnesene	1.8 ± 0.1	ND	0.5 ± 0.2
1460	Alloaromadendren	ND	ND	0.1 ± 0.1
1488	α-Curcumene	ND	4.0 ± 0.1	ND
1482	Germacrene D	3.4 ± 0.1	11.9 ± 0.4	0.2 ± 0.1
1492	Varidiflorene	ND	ND	0.2 ± 0.1
1485	β-Selinene	ND	0.7 ± 0.0	Tr
1520	γ-Cadinene	0.7 ± 0.2	2.0 ± 0.1	0.1 ± 0.0
1529	(-)-Calamenene	3.2 ± 0.1	4.2 ± 0.2	ND
1544	Selina-3,7(11)-diene	ND	0.8 ± 0.0	ND
1547	Elemol	ND	0.1 ± 0.0	ND
1596	Guaiol	ND	ND	0.2 ± 0.1
1584	Caryophyllene oxide	ND	0.3 ± 0.0	0.7 ± 0.1
1587	Ledol	0.4 ± 0.0	0.4 ± 0.0	0.5 ± 0.2
1617	Cubenol	0.7 ± 0.0	0.5 ± 0.0	ND
1649	γ-Eudesmol	ND	0.7 ± 0.0	ND
1660	α-Cadinol	ND	0.2 ± 0.0	0.1 ± 0.0
1674	Cadalene	0.1 ± 0.0	0.1 ± 0.0	ND
1574	Spathulenol	ND	0.1 ± 0.0	ND

Table 1. (Continued)

RI[z]	Compound	Relative composition (%)		
		Spearmint	Apple mint	Chocolate mint
	Esters			
717	Propyl acetate	7.9 ± 0.6	ND	0.4 ± 0.1
848	Ethyl-2-methyl butyrate	0.1 ± 0.0	0.5 ± 0.1	ND
1108	1-Octen-3-yl-acetate	0.5 ± 0.2	0.4 ± 0.0	ND
1129	3-Octyl acetate	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
1287	Menthyl acetate	ND	0.4 ± 0.0	0.1 ± 0.0
1387	Geranyl acetate	ND	ND	0.4 ± 0.1
1344	Dihydrocarveol acetate	0.6 ± 0.4	0.2 ± 0.0	ND
1342	(*E*)-Carvylacetate	0.1 ± 0.0	ND	ND
1350	α-Terpineol acetate	ND	ND	1.1 ± 0.4
1362	(-)-cis-Carvyl acetate	0.4 ± 0.1	ND	Tr
1497	Phenethyl isovalerate	ND	ND	0.5 ± 0.2
1827	Benzyl salicylate	ND	ND	0.1 ± 0.0
	C6 compounds			
854	(*E*)-2-Hexenal	0.1 ± 0.0	0.4 ± 0.0	ND
857	(*Z*)-3-Hexene-1-ol	0.2 ± 0.0	ND	ND
862	(*E*)-2-Hexen-1-ol	0.2 ± 0.2	ND	ND
1003	(*Z*)-3-Hexenylacetate	0.3 ± 0.0	ND	ND
	Alcohols			
982	1-Octen-3-ol	0.1 ± 0.0	5.8 ± 0.3	ND
995	3-Octanol	0.2 ± 0.1	0.6 ± 0.0	0.2 ± 0.1
	Aldehydes			
1045	Benzeneacetaldehyde	0.7 ± 0.2	1.5 ± 0.8	0.3 ± 0.2
1250	*p*-Anisaldehyde	ND	ND	0.2 ± 0.2
1606	Tetradecanal	ND	0.1 ± 0.0	ND
	Ketones			
1394	(*Z*)-Jasmone	ND	0.3 ± 0.1	0.1 ± 0.0
1922	Farnesyl acetone	0.1 ± 0.0	0.2 ± 0.0	ND
	Fatty acids			
1968	*n*-Hexadecanoic acid	0.1 ± 0.0	0.6 ± 0.0	0.3 ± 0.1
2116	Linolenic acid	0.5 ± 0.1	1.9 ± 0.1	ND

[z]Retention index based on a series of *n*-hydrocarbonsfrom DP-5MS column.
[y]Values are given as the mean ± standard deviation of three replications of each sample.
ND = not detected; Tr = trace amount (less than 0.1%).

only in spearmint, 8 only in apple mint and 20 specifically in chocolate mint. On the basis of the relative proportion of the primary classes, monoterpenoids comprised the major compounds in all species followed by sesquiterpenoids: spearmint (monoterpenoids, sesquiterpenoids; 75.0%, 12.6%), apple mint (58.0%, 29.0%) and chocolate mint (92.6%, 3.5%) (Table 1). In particular, (-)-carvone (33.0%), R-(+)-limonene (11.7%) and β-phellandrene (9.7%) in spearmint; (-)-carvone (37.4%) and germacrene D (11.9%) in apple mint; and (-)-menthol (34.3%), *p*-menthone (18.4%) and menthofuran (9.8%) in chocolate mint were found as the major compounds in each mint species. These results were matched to the previous reports: carvone and limonene in spearmint and (-)-menthol and (-)-menthone in chocolate mint as major compounds (Barton et al., 1992; Benyoussef et al., 2005; Miyazawa et al., 1998). Carvone was also reported as major compounds in

Table 2. Chemical composition (%) of primary classes of the essential oils in three *Mentha* Species by GC-MS Analysis.

Class	Relative composition (%)		
	Spearmint	Apple mint	Chocolate mint
Total monoterpenoids	75.0 ± 1.4[z]	58.0 ± 0.6	92.6 ± 2.0
Total sesquiterpenoids	12.6 ± 0.4	29.0 ± 0.8	3.5 ± 0.8
Total esters	9.7 ± 0.6	1.6 ± 0.1	2.7 ± 0.8
Total C6 compounds	0.8 ± 0.2	0.4 ± 0.0	ND
Total alcohols	0.3 ± 0.1	6.4 ± 0.4	0.2 ± 0.1
Total aldehydes	0.7 ± 0.2	1.6 ± 0.8	0.5 ± 0.3
Total ketones	0.1 ± 0.0	0.5 ± 0.1	0.1 ± 0.0
Total fatty acids	0.6 ± 0.1	2.5 ± 0.1	0.3 ± 0.1

[z]Values are given as the mean ± standard deviation of three replications of each sample.
ND = not detected.

dill and caraway, showing antimicrobial activity (Bailer et al., 2001). Menthol was reported to have significant antifungal activity (Sokovic et al., 2006). In addition to terpenoids including monoterpenoids and sesquiterpenoids, esters (9.7%) and C6 compounds (0.8%) were quantitatively the major compounds in spearmint while alcohols (6.4%) and fatty acids (2.5%) in apple mint, esters (2.7%) and aldehydes (0.5%) in chocolate mint.

Multivariate Analyses of Essential Oil Compounds in Mint

Three different mint species (i.e., spearmint, apple mint and chocolate mint) were clearly separated using HCA on the basis of 91 essential oil compounds (Fig. 1). Especially, essential oil composition in chocolate mint was quite different from that in spearmint and apple mint. PCA supported the result obtained from HCA in that the chemical compositions of spearmint, apple mint and chocolate mint were clearly separated, accounting for 95.6% of the total variance by combining the PC 1 (principal component) and second (PC 2) (Fig. 2). Chocolate mint positioned on the positive side of PC 1, were clearly separated from spearmint and apple mint positioned on the negative side of PC 1, accounting for 87.6% of the total variance. (-)-Menthol and *p*-menthone were the most important contributor influencing the separation of chocolate mint from spearmint and apple mint. Apple mint was also clearly separated from spearmint on the basis of PC 2. (-)-Carvone was the most important contributor to the difference in apple mint from the other species; R-(+)-limonene, propyl acetate and β-phellandrene to the difference in spearmint from the others. However, the separation of spearmint and apple mint on the basis of PC 2 is very weak due to very low eigenvalue of PC 2 (8.0% of the total variance). The results represent that the three mint species have the different chemical composition in the essential oils from their aerial parts, showing chocolate mint has very distinctive essential oil type from other mint species. Three different mint species

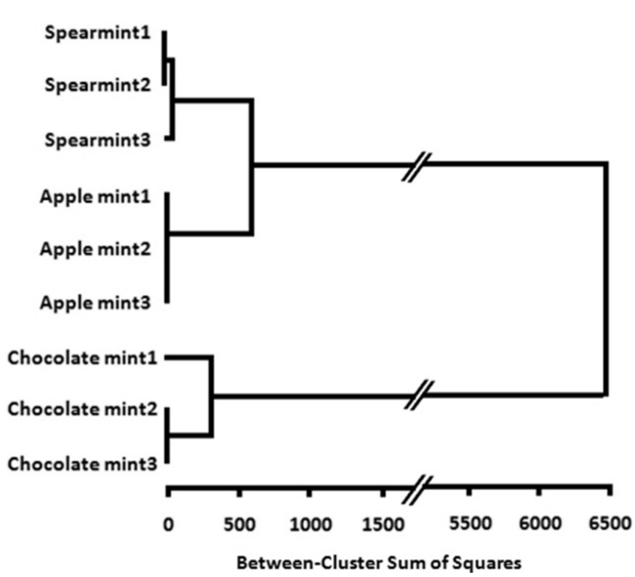

Fig. 1. Hierarchical cluster analysis using 91 essential oil compounds from spearmint, apple mint and chocolate mint.

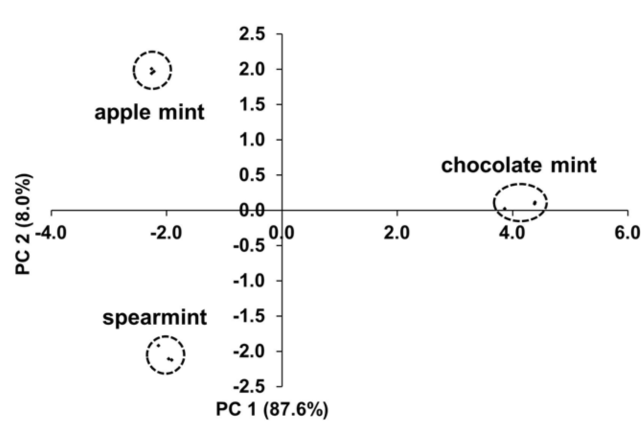

Fig. 2. Principle components analysis plot using 91 essential oil compounds from spearmint, apple mint and chocolate mint.

are expected to have a variety of biological activity due to the variation in essential oil composition.

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