

Chemical characterization of basil (*Ocimum* spp.) based on volatile oils

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ABSTRACT: Essential oils extracted from basil (*Ocimum* spp.) by steam distillation are used to flavor foods, oral products, in fragrances and in traditional medicines. The genus *Ocimum* contains around 30 species native to the tropics and subtropics, with some species naturalized and/or cultivated in temperate areas. Inter- and intraspecific hybridization have created significant confusion in the botanical systematics of the *Ocimum* genus. The taxonomy of *O. basilicum* is further complicated by the existence of numerous varieties, cultivars and chemotypes within the species that do not differ significantly in morphology. The composition of volatile oil constituents was used to characterize the diversity among the most economically important *Ocimum* species. Using principal component analysis on the aromatic volatile oils, the *Ocimum* accessions could be separated into five groups, which do not correspond to the different species: (1) citral–spathulenol accessions; (2) linalool-rich accessions; (3) methylchavicol-rich accessions; (4) linalool–methylchavicol accessions; and (5) methyl(*E*)-cinnamate-rich accessions. The fact that the groups of *Ocimum* species are based on morphological characteristics does not correspond to the groups based on volatile oil constituents. Copyright © 2005 John Wiley & Sons, Ltd.

KEY WORDS: *Ocimum*; *O. basilicum*; *O. × citriodorum*; *O. americanum* var. *americanum*; *O. americanum* var. *pilosum*; *O. minimum*; *O. kilimandscharicum*; essential oils; infraspecific variability, chemotaxonomy

Introduction

Basil (*Ocimum basilicum* L.) leaves are used fresh and dried as flavorings or spices in sauces, salad dressings, vegetables, vinegar and confectionery products.¹ Basil is also an important ornamental plant, with a variety of leaf and inflorescence shapes, sizes and colors. Besides the commercial basil, many other species of *Ocimum* are used in traditional ceremonies and medicines, mainly in their country of origin.^{2–4} Extracted essential oils from basil are used to flavor foods and dental and oral products.¹ Basil volatile oils have also been shown to contain biologically active constituents that are insecticidal, nematocidal, fungistatic and have antimicrobial properties.⁵

More than 40 types of basil available in North America⁶ have been described. Most varieties available and used belong to *O. basilicum*, the most cultivated species. Darrah⁷ classified *O. basilicum* varieties into four groups: (1) tall slender types, which included the variety ‘Sweet Basil’; (2) large-leaved, robust types, such as the

variety ‘Lettuce Leaf’; (3) dwarf types, small-leaved, including the variety ‘Bush’ basil; and (4) compact types, described as a botanical variety, *O. basilicum* var. *thyrsiflora*, commonly called Thai basil. Later, Darrah⁸ included three additional groups: (5) *O. basilicum* var. *purpurascens*, the purple basil types with a sweet flavor; (6) dark opal type, a possible hybrid between *O. basilicum* and *O. forskolei*, which has lobed purple leaves with sweet basil plus clove flavor; and (7) *citriodorum*-type, including lemon flavor basil.

Basil (*O. basilicum*) varieties have been selected and developed over many years for a variety of purposes. The culinary basil varieties were selected for their leaf shape, size, aroma and flavor. In the sweet basil group are many green basil varieties, rich in a combination of linalool, methylchavicol and 1,8-cineole. The most important ones used in fresh culinary herbs include ‘Italian Large Leaf’, ‘Genovese’, and ‘Sweet basil’. Basil varieties were also selected and modified to enhance their ornamental qualities, focusing on such traits as plant height, leaf color and shape, inflorescence length, color and compactness. Examples include the varieties ‘Bush’, selected for its dwarf form, ‘Purple Ruffles’, selected for its leaf color, ‘Siam Queen’ for its beauty and ‘Sweet Dani Lemon Basil’ for its citral aroma and large leaf. Centuries of selection of basil for distinct aromas also led to the establishment of many chemotypes rich in a variety of volatile oil compounds, including citral, linalool, methylchavicol, eugenol, geraniol and methylcinnamate.

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Several types of basil are also produced for their extractable essential oils, which are traded in the international essential oil market, each derived from different cultivar or chemotype of *O. basilicum*, and classified according to their geographic origin.¹⁹ The European type (Italy, French or sweet basil), considered to be the highest quality and producing the finest odor, contains linalool and methylchavicol as the major constituents.¹ Egyptian basil is very similar to the European, but with a higher relative percentage of methylchavicol. The Reunion type, from the Reunion and Comoro Islands, Thailand, Madagascar and Vietnam, is characterized by its high concentrations of methylchavicol.⁹ Methylcinnamate-rich basil has been commercially produced in Eastern Europe,¹ India, Guatemala and Pakistan.⁹ A basil from Java,¹ and Russia and North Africa⁹ has been reported to be a eugenol-rich basil.

The occurrence of interspecific hybridization and polyploidy within this genus¹⁰ of about 30 species¹¹ has created taxonomic confusion and challenges to understanding the genetic relationship between the multitude of basil species.¹² Taxonomy of basil (*O. basilicum*) is also complicated by the existence of numerous varieties, cultivars and chemotypes within the species that do not differ significantly in morphology.¹ Hybridization of many different species and varieties, and the widespread use of commercial variety names, have obscured important information such as variation in chemistry and chromosome number. Paton and Putievsky¹³ reported that there is no clear morphological discontinuity between *O. basilicum* varieties.

A system of standardized descriptors, including volatile oil, was proposed by Paton and Putievsky¹³ to allow easy communication and correct identification of the different forms of *O. basilicum*. Molecular markers using RAPD have been used successfully in assessing genetic diversity and distinguishing genetic relatedness among a wide number of *Ocimum* spp.^{14,15}

Principal component analysis (PCA) has been successfully used in many aromatic and polymorphic species, such as *Mentha* and *Thymus*,^{16–18} and has been associated with RAPD markers.^{14,15,19,20} Our results with *O. gratissimum*¹⁴ showed that the use of principal component analysis can be an important tool for working on multivariate data.

The objective of this research was to chemically characterize the volatile oil constituents in an *Ocimum* germplasm collection and to assess the relative use of this parameter as a taxonomical descriptor.

Material and Methods

Plant Material

Twenty-six accessions (Table 1) of *Ocimum basilicum*

(15), *O. americanum* var. *americanum* (2), *O. americanum* var. *pilosum* (1), *O. minimum* (3), *O. × citriodorum* (4), and one hybrid between *O. basilicum* and *O. kilimandscharicum* [obtained from Richters Seed Co., Goodwood, Ontario, Canada; Companion Plants, Athens, OH, USA; Purdue University, West Lafayette, IN, USA; Kew Gardens, London, UK; US Department of Agriculture (USDA), Plant Introduction Station, Ames, IA, USA; Brazilian Agricultural Research Corporation (Embrapa), Brasília, Brazil; and Nichols Garden, Albany, OR, USA] were grown at Purdue University Research Station, West Lafayette (IN, USA) in a randomized complete-block experimental design, with four plants in each of the three replications. The above-ground biomass of each individual plant from each block was harvested at full bloom, bulked, placed in a paper bag and dried in a forced-air drier at 38 °C for 15 days for oil analysis. Voucher specimens of each accession included in our experiments were collected, dried and stored at the Ralph Kriebel herbarium (PUL), Purdue University. The taxonomic identification was conducted in collaboration with Dr A. Paton from the Royal Botanical Garden, Kew, London, Dr A. Tucker from Delaware State University and Dr U. Albuquerque from Universidade Federal de Pernambuco, Brazil. Voucher specimens were sent to Kew Gardens (K), Delaware State University (DOV) and Cenargen, Embrapa (CEN) herbaria for permanent collection.

Volatile Oil Analysis

The volatile oil was extracted from dried samples in a 2 l flask with distilled water (1:15 w/v) using a Clevenger apparatus, as previously described.²¹ Volatile oil samples were analyzed in a Varian 3700 gas chromatograph equipped with a flame ionization detector (FID) and an electronic 4270 integrator. A fused silica capillary column (30 m × 0.25 mm i.d.) with an RSL-200 (Altech, 5% phenyl, 95% methylpolysiloxane) bonded phase was used. Helium was used as a carrier gas and the oven temperature was held isothermal at 80 °C for 2 min and then programmed to increase at 3 °C/min to 160 °C. This gave a complete elution of all peaks (using a sensitivity of 10⁻¹⁰, attenuation 16). The injector and detector temperatures were 180 and 300 °C, respectively. Standards samples of oil constituents (*p*-cymene, 1,8-cineole, terpinen-4-ol, methylchavicol, geraniol, thymol, eugenol, methyleugenol and β -caryophyllene) were run at the same conditions. Identification of essential oil constituents was confirmed by GC/MS using a Finningan 4000; the carrier gas was helium with a column pressure of 10.5 psi and split vent of 40 ml/min; the oven program was 80 °C for 2 min and then increasing at 3 °C/min to 160 °C; injection temperature was 225 °C. The MS conditions include ionization voltage, 70 eV; emission

current, 40 mA; scan rate, 1 scan/s; mass range, 40–500 Da; ion source temperature, 160 °C.²¹

Data Analysis

Statistical analysis of volatile oil constituents was performed using SAS software (SAS Institute Inc., Cary, NC, USA) procedures. Principal component analysis (PCA) was performed to display the relationship among accessions in terms of their position relative to three coordinate axes. All PCA was carried out using SAS (SAS Institute Inc., Cary, NC, USA) software procedures, and drawn using Sigma Plot (Jandel Corporation, San Rafael, CA, USA).

Results and Discussion

Twenty-seven volatile oil constituents (Tables 1–5) were identified across the 26 *Ocimum* accessions (Table 1).

The essential oil content varied from 0.27% in *O. americanum* var. *pilosum* (ot21) to 2.47 and 2.8% in *O. americanum* var. *americanum* (ot5) and ‘African blue’ (ot50), respectively. The shortest plants were from *O. minimum* (ot34, ot35 and ot60), each reaching heights of only 28.3, 24.8 and 30.8 cm, respectively. In contrast, plants of *O. basilicum* reached heights of from 33.8 cm in ‘Green Ruffles’ (ot41) to 72.3 cm in ‘Sweet Dani Lemon Basil’ (ot70).

Essential oil content in *O. × citriodorum* ranged (as a percentage dry weight) from 0.36 (ot58) to 0.39% (ot53) to 1.64 and 1.12%, for ‘Lemon Mrs. Burns’ (ot61) and ‘New Guinea’ (ot62), respectively (Table 1). The ratios of total volatile oil in ‘Sweet Dani’ (ot70) and ‘Lemon Mrs. Burns’ (ot61), both considered *O. basilicum* with strong lemon ancestry basil types,¹¹ were consistent with the literature.

‘Sweet Dani Lemon Basil’ (ot70) contained the highest citral concentration (33.6%); however, the relative amount was much lower than the 68% citral reported by Morales and Simon.²² Grayer *et al.*²³ reported a citral

Table 1. List of *Ocimum* accessions, origin, growth and essential oil accumulation, grown at Purdue University (West Lafayette, IN, USA)

Species accessions	Origin	Height (cm)	Total fresh weight (g)	Total dry weight (g)	Essential oil (% dry weight)
<i>O. americanum</i> var. <i>americanum</i>					
ot5 ^a	Kew90157, Kew Garden	45.3 ± 1.7 ^b	502.3 ± 117	112.2 ± 25	2.47 ± 0.2
ot32	Brasilia, Brazil	34.3 ± 2.4	266.3 ± 17	69.8 ± 4	1.68 ± 0.4
<i>O. americanum</i> var. <i>pilosum</i>					
ot21	PI414204, USDA	46.3 ± 3.8	494.5 ± 70	112.4 ± 16	0.27 ± 0.1
<i>O. basilicum</i>					
ot2	kew7711, Kew Garden	48.5 ± 1.3	301 ± 116	79 ± 28	0.87 ± 0.2
ot38	Dark Opal, Richters Co.	44.8 ± 3.6	391.3 ± 77	59.7 ± 12	1.1 ± 0.2
ot40	Purple Ruffles, Richters Co.	34.8 ± 2.6	218.8 ± 50	30.1 ± 7	0.58 ± 0.4
ot41	Green Ruffles, Richters Co.	33.8 ± 5.3	490.8 ± 194	68.6 ± 25	0.83 ± 0.2
ot43	Mammoth, Richters Co.	42 ± 9.1	672.3 ± 345	106.8 ± 56	0.75 ± 0.2
ot44	Sweet, Richters Co.	49 ± 5.6	544.5 ± 255	94.6 ± 45	1.12 ± 0.2
ot45	Sweet Fine, Richters Co.	45.5 ± 0.7	403.5 ± 103	71 ± 13	0.77 ± 0.3
ot49	Cinnamon, Richters Co.	46.5 ± 5.2	554.3 ± 163	97.2 ± 65	1.54 ± 0.3
ot56	Thai, Richters Co.	38 ± 3.3	538.3 ± 164	96.5 ± 24	0.71 ± 0.1
ot57	Thai Siam Queen, Richters Co.	38.3 ± 5.6	544.3 ± 58	93.6 ± 3	0.70 ± 0.1
ot61	Lemon Mrs Burns, Companion	59 ± 5.4	754.3 ± 272	150.1 ± 59	1.64 ± 0.2
ot69	Comoro, Purdue	61 ± 1.4	697.9 ± 205	168.3 ± 35.6	1.04 ± 0.3
ot70	Sweet Dani Lemon Basil, Purdue	72.3 ± 4.0	761.5 ± 132	176 ± 38.4	0.54 ± 0.1
ot71	Methyl(<i>E</i>)-cinnamate basil, Purdue	38.9 ± 3	462.8 ± 122	102.9 ± 25.3	1.37 ± 0.3
ot72	Linalool basil, Purdue	58.5 ± 3.3	638.2 ± 236	137.4 ± 46.2	1.13 ± 0.3
<i>O. basilicum</i> cv. <i>Dark Opal</i> × <i>O. kilimandscharicum</i>					
ot50	African Blue, Richters Co.	56.3 ± 1.7	636.3 ± 185	141.6 ± 38.2	2.8 ± 0.1
<i>O. × citriodorum</i>					
ot53	Lemon, Richters Co.	46 ± 2.2	354.5 ± 109	93.2 ± 29.3	0.39 ± 0.2
ot58	Lemon, Companion	48.8 ± 3.2	499.2 ± 117	109.1 ± 18.4	0.36 ± 0.1
ot62	New Guinea, Companion	32 ± 2.5	303 ± 43	64.2 ± 12.2	1.12 ± 0.4
ot66	Lemon, Nichols Garden Nursery	45.5 ± 4.0	406.8 ± 143	99.9 ± 35.9	0.38 ± 0.1
<i>O. minimum</i>					
ot34	Bush, Richters Co.	28.3 ± 2.6	471 ± 103	82 ± 11.4	1.06 ± 0.2
ot35	Spicy globe, Richters Co.	24.8 ± 5.0	433 ± 167	64.1 ± 20	1.39 ± 0.1
ot60	Dwarf Opal, Companion	30.8 ± 2.2	449 ± 150	69.7 ± 22.4	1.05 ± 0.4

^a ot = *ocimum* accessions numbers.

^b All values reported as an average of three replicates ± SD.

Table 2. Chemical composition of *Ocimum americanum* accessions, grown under field conditions (Purdue University)

Volatile oil constituents ^a	RT ^b	Var. <i>americanum</i>		Var. <i>pilosum</i>
		ot5	ot32	ot21
<i>(Relative percentage of total volatile oil)^c</i>				
1,8-Cineole	6.08	25.6 ± 6.3		15.5 ± 12.8
Linalool	8.1	4.7 ± 1		1.7 ± 1.1
Camphor	9.24	1.4 ± 0.5		1.05 ± 0.2
Methylchavicol	11.72	0.4 ± 0.3		10.4 ± 5.3
Anisole	14.08	42.9 ± 5.7		
Methyl(Z)-cinnamate	16.68		9.65 ± 0.4	
Eugenol	18.72	0.4 ± 0.3		1.6 ± 0.3
Methyl(E)-cinnamate	20.86		86.5 ± 0.7	0.6 ± 0.3
β-Caryophyllene	20.58	1.5 ± 0.6		0.7 ± 0.4
α-Bergamotene	21.24			5.2 ± 2.4
α-Humulene	22.04	11.5 ± 4		1.8 ± 0.5
β-Bisabolene	24.58			29.2 ± 9.2
α-Farnesene	25.74			1.6 ± 0.5
Spathulenol	27.6			5.8 ± 2.2

^a Listed in order of retention time (min).^b RT = retention time.^c Values reported as an average of three replicates ± SD.ot5 = *O. americanum* (Kew), ot32 = *O. americanum* (Brazil).

concentration of 78% in *O. × citriodorum* using solvent extraction method. Extraction methods for citral can lead to significant differences in citral recovery.

Ocimum americanum var. *americanum* (ot32 and ot5) exhibited the highest volatile oil yield per plant (1.68 and 2.47%, respectively), while the *O. americanum* var. *pilosum* (ot21) produced the lowest oil (0.27%; Table 1). *Ocimum basilicum* accessions exhibited a range in oil production from 0.58 to 1.64%.

Comparing the volatile oil constituents between the different varieties of *O. americanum* (Table 2), *O. americanum* var. *americanum* and *O. americanum* var. *pilosum* showed a completely distinct oil profile. We observed two different chemotypes in the first variety, anisole-rich (ot5) and a methylcinnamate-rich (ot32) chemotypes. The botanical variety *pilosum* contained β-bisabolene and methylchavicol as its major constituents. This is the first time *O. americanum* var. *pilosum* volatile oil has been chemically described, but more accessions will have to be studied to see whether this is a characteristic essential oil profile for var. *pilosum*.

Ocimum americanum appears to be the most chemically variable species in the genus, with four chemotypes described in the literature. Distinct chemotypes of *Ocimum americanum* have been reported, such as a citral type in India,²⁴ a camphor type from Africa,²⁵ and a methyl(E)-cinnamate chemotype in Brazil.⁴ Examination of a methyl(E)-cinnamate chemotype of *O. americanum* showed no linkage between morphology and chemical compounds, since other methyl(E)-cinnamate types were also found in *O. basilicum*.

A total of 21 volatile oil constituents were detected in *O. basilicum* (Table 3). The major constituents

detected in our samples included 1,8-cineole, linalool, methylchavicol and methyl(E)-cinnamate, all previously described.²⁶

The analysis of *O. basilicum* accessions showed mostly a variation of linalool–methylchavicol types, with only two accessions (ot49 and ot71; Table 4) rich in methyl(E)-cinnamate. Two additional accessions of *O. basilicum* (ot66 and ot70; Table 3) were considered distinct from other *O. basilicum* accessions. Although morphologically described as *O. basilicum*, ‘Sweet Dani Lemon Basil’ (ot70) and ‘Lemon Mrs Burns’ (ot66) exhibited an oil profile similar the generic lemon basil (*O. × citriodorum*), and thus appeared to be chemically closer to *O. × citriodorum*. These findings are supported by genetic fingerprinting of this species using RAPDs, which placed all citral-rich basil as *O. × citriodorum* rather than as *O. basilicum*.¹⁵

A detailed analysis of *O. basilicum* varieties, showed that ‘Cinnamon’ basil (ot49) and the Purdue methyl(E)-cinnamate chemotype (ot71) can be differentiated by the amount of methyl(E)-cinnamate in their oil, with 34 and 82%, respectively. ‘Cinnamon’ basil appears to have been introduced to USA from Mexico, as ‘Spicy Mexican’ basil, and was the first flavored variety to be sold by a large US seed company.⁶ The methyl(E)-cinnamate chemotype (ot71) was developed over a 10 year breeding project by Purdue University at the Center for New Crops and Plant Products.

The *O. basilicum* accessions ‘Dark Opal’ (ot38) and ‘Purple Ruffles’ (ot40) showed similar volatile oil profiles, although the first exhibited a higher linalool content, and the second a higher methylchavicol content. ‘Purple Ruffles’ is a cross between ‘Dark Opal’ and

Table 3. Chemical composition of *Ocimum basilicum* accessions, grown under field conditions (Purdue University)

Volatile oil constituents ^a	RT ^b	ot2 ^c	ot38	ot40	ot41	ot43	ot44	ot45
	(Relative percentage of total volatile oil) ^d							
α -Pinene	4.79		1.1 \pm 0.7	1.7 \pm 1.6	1.5 \pm 0.7	0.6 \pm 0.4	0.4 \pm 0.1	0.1 \pm 0
β -Pinene	4.96		0.8 \pm 0.47	1.9 \pm 1.5	1.5 \pm 0.5	0.4 \pm 0.1	0.4 \pm 0.17	0.3 \pm 0.14
1,8-Cineole	6.08	1.4 \pm 0.76	11 \pm 4	14.7 \pm 8.5	14.8 \pm 2.3	7.8 \pm 4.4	8.4 \pm 1.8	2 \pm 1.1
γ -Terpineol	7.31	0.5 \pm 0.6	0.9 \pm 0.43	0.9 \pm 0.81	0.5 \pm 0.3	1 \pm 0.3	0.9 \pm 0.52	0.8 \pm 0.42
Linalool	8.1	0.6 \pm 0.8	69.3 \pm 5.6	38.1 \pm 8.2	20.3 \pm 3.4	34.1 \pm 5.6	55.7 \pm 19.3	70.6 \pm 6.2
Camphor	9.24				2.9 \pm 0.3	0.2 \pm 0.3	0.3 \pm 0.32	
Terpin-4-ol	10.77				0.5 \pm 0.2	0.4 \pm 0.3	0.6 \pm 0.57	0.2 \pm 0.21
Methylchavicol	11.72	87.8 \pm 5.8	1.3 \pm 0.3	12.7 \pm 18.6	41.5 \pm 2.2	41.7 \pm 5.5	16.4 \pm 20.1	2.6 \pm 0.21
Methyl(Z)-cinnamate	16.68							
Eugenol	18.72		0.6 \pm 0.29	0.4 \pm 0.4	0.3 \pm 0.2	0.1 \pm 0.1	1.8 \pm 3.1	3.5 \pm 1.3
β -Elemene	19.44	0.5 \pm 0.25	1 \pm 0.26	1.9 \pm 1.6	0.7 \pm 0.2	0.9 \pm 0.3	1.1 \pm 0.6	0.9 \pm 0.7
Methyl(E)-cinnamate	20.45							
Methyleugenol	20.58	0.9 \pm 0.92	0.6 \pm 0.06	1.1 \pm 0.5	0.2 \pm 0.1	0.1 \pm 0.1	0.2 \pm 0.08	0.2 \pm 0
β -Caryophyllene	20.86	1.3 \pm 1.14						
γ -Elemene	21.24	1.3 \pm 0.4	1.6 \pm 0.92	10.9 \pm 3.9	3.6 \pm 0.5	2.4 \pm 0.68	1.6 \pm 0.65	4.4 \pm 2.5
α -Humulene	22.04		0.5 \pm 0.1	1.1 \pm 0.6	0.6 \pm 0.1	0.4 \pm 0.15	0.5 \pm 0.27	0.6 \pm 0.1
β -Cubebene	23.2		0.8 \pm 0.34	1.8 \pm 0.6	1.4 \pm 0.1	1.4 \pm 0.26	1 \pm 0.5	1.2 \pm 0.6
β -Bisabolene	24.58		1.2 \pm 0.5	1.7 \pm 0.2	1.4 \pm 0.2	1.5 \pm 0.3	1.7 \pm 0.57	1.6 \pm 0.5
α -Farnesene	25.74							
α -Gurjenene	26.66							
Spathulenol	27.6	1 \pm 0.7	1.0 \pm 0.32	1.3 \pm 0.2	0.3 \pm 0.1	0.5 \pm 0.06	0.9 \pm 0.6	1.1 \pm 0.35
α -Pinene	4.79	0.3 \pm 0.26	0.2 \pm 0.06					
β -Pinene	4.96	0.3 \pm 0.32	0.1 \pm 0.08					
1,8-Cineole	6.08	2.9 \pm 1.6	1.7 \pm 0.66	0.9 \pm 0.65	0.3 \pm 0.19	3.7 \pm 2.7	0.7 \pm 0.38	
γ -Terpineol	7.31	2.1 \pm 1.7	0.7 \pm 0.46	t	0.5 \pm 0.54	0.6 \pm 0.25	2.6 \pm 1.54	
Linalool	8.1	32.3 \pm 13.1	1.4 \pm 0.6	0.8 \pm 0.15	0.6 \pm 0.36	1.7 \pm 0.49	74.5 \pm 1.7	
Camphor	9.24	0.9 \pm 0.28	4.3 \pm 0.88	3 \pm 2.04				
Terpin-4-ol	10.77	0.8 \pm 0.32	1 \pm 0.29	0.8 \pm 0.53			0.3 \pm 0.1	
Methylchavicol	11.72	6.4 \pm 10.8	74.4 \pm 3.4	57.2 \pm 38.2	86.4 \pm 3.8	0.4 \pm 0.1	0.4 \pm 0.15	
Methyl(Z)-cinnamate	16.68	5.5 \pm 3.7				5.9 \pm 0.75		
Eugenol	18.72	1 \pm 2			0.1 \pm 0.2		0.8 \pm 0.62	
β -Elemene	19.44		0.3 \pm 0.3		0.8 \pm 0.48		1.2 \pm 0.22	
Methyl(E)-cinnamate	20.45	34 \pm 22.8	1.0 \pm 0.13	0.8 \pm 0.55		82.4 \pm 4.2		
Methyleugenol	20.58				2.5 \pm 0.75			
β -Caryophyllene	20.86							
γ -Elemene	21.24	0.4 \pm 0.8	4.5 \pm 0.64	3.1 \pm 2.08	0.8 \pm 0.29		1.9 \pm 0.3	
α -Humulene	22.04	0.3 \pm 0.6	0.2 \pm 0.1	0.3 \pm 0.41	0.8 \pm 0.24		0.3 \pm 0.21	
β -Cubebene	23.2	1.7 \pm 2.26	0.5 \pm 0.22	0.7 \pm 0.95	1.7 \pm 0.52		2.3 \pm 0.74	
β -Bisabolene	24.58	1.9 \pm 1.12	1.4 \pm 0.21	0.9 \pm 0.65	0.2 \pm 0.2	0.2 \pm 0.25	2.5 \pm 0.56	
α -Farnesene	25.74		1.3 \pm 0.1	1 \pm 0.73	2 \pm 0.69			
α -Gurjenene	26.66	1.1 \pm 0.78	0.2 \pm 0.21		0.1 \pm 0.2			
Spathulenol	27.6	1.3 \pm 0.27	1.6 \pm 0.58	1.2 \pm 1	0.7 \pm 0.22	0.9 \pm 0.42	0.9 \pm 0.1	

^a Listed in order of retention time (min); ^b RT = retention time.

^c ot2 = *O. basilicum* (Kew); ot38 = 'Dark Opal'; ot40 = 'Purple Ruffles'; ot41 = 'Green Ruffles'; ot43 = 'Mammoth'; ot44 = 'Sweet'; ot45 = 'Sweet Fine'; ot49 = 'Cinnamon'; ot56 = 'Thai'; ot57 = 'Thai Siam Queen'; ot69 = 'Comoro'; ot71 = 'Purdue methyl(E)-cinnamate'; ot72 = 'Purdue linalool'.

^d Values reported as an average of three replicates \pm SD.

'Green Ruffles' (ot41), which also showed high levels of methylchavicol (41.5%).

The constituent profile in culinary basil is a very important character. Among the green basil, 'Green Ruffles' (ot41), 'Mammoth' (ot43), 'Sweet' (ot44) and 'Sweet Fine' (ot45) contained 1,8-cineole, linalool and methylchavicol as the major aromatic compounds. The ratio of 1,8-cineole–linalool–methylchavicol varied by variety (15:20:42, 8:34:42, 8:56:16 and 2:70:3, respectively), contributing to their particular aromas.

Linalool chemotype (ot72) had distinct leaf shape compared with 'Sweet Fine' (ot45), although both are linalool-rich. The varieties 'Thai' (ot56), 'Thai Siam Queen' (ot57) and 'Comoro' (ot69) had the highest levels of methylchavicol. One accession introduced from

the Royal Botanical Garden at Kew, UK (ot2), and also a methylchavicol type, was morphologically distinct from all other methylchavicol basil.

Of the *O. × citriodorum* accessions (Table 5) analyzed, including 'Lemon Mrs Burns' (ot61) and 'Sweet Dani Lemon Basil' (ot70), all showed citral as their major constituent, except for the New Guinea (ot62), a methylchavicol type with no citral. 'Lemon Mrs Burns' (ot61) differs from the others citral types by its high linalool content (46%).

Of the three accessions of *O. minimum*, 'Bush' (ot34), 'Spice Globe' (ot35) and 'Dwarf Opal' (ot60; Table 5), the first two are green dwarf varieties, while the third is a dwarf purple. All contained linalool as their major constituent (46, 46 and 67%, respectively), followed by

Table 4. Chemical composition of *Ocimum* × *citriodorum* accessions, and lemon-scented *O. basilicum*, grown under field conditions (Purdue University)

Volatile oil constituents ^a	RT ^b	ot53 ^c	ot58	ot61	ot62	ot66	ot70 ^d
(Relative percentage of total volatile oil) ^e							
β-Pinene	4.96	0.1 ± 0.1	0.3 ± 0.16	0.5 ± 0		0.23 ± 0.1	0.55 ± 0.06
1,8-Cineole	6.08	0.5 ± 0.4	0.5 ± 0.34	2.4 ± 0.68	2.4 ± 0.5	0.4 ± 0.19	0.4 ± 0.15
γ-Terpineol	7.31	1.6 ± 0.13	2.1 ± 0.39	3.4 ± 0.3		2 ± 0.24	1.6 ± 0.61
Linalool	8.1	8.9 ± 1	10.7 ± 1	46.1 ± 3.9	0.6 ± 0.59	10.2 ± 1	1.1 ± 0.4
Camphor	9.24	0.3 ± 0.17	0.3 ± 0.19	0.6 ± 0.16		0.2 ± 0.24	0.5 ± 0.1
Terpin-4-ol	10.77	0.6 ± 0.32	0.6 ± 0.13	0.5 ± 0.06	1 ± 0.1	0.6 ± 0.06	0.7 ± 0.05
Methylchavicol	11.72	3.7 ± 0.68	3.7 ± 0.22	6.4 ± 0.27	84 ± 3.6	4.3 ± 0.75	4.4 ± 2
Citral (neral + geranial)	13.2 + 14.4 ^f	31.3 ± 4.2	29 ± 1	16.6 ± 2.6		32.3 ± 8.5	33.6 ± 2.4
Thymol	16.42	3 ± 0.6	5.5 ± 1.5	2.4 ± 0.9		2.9 ± 1	4.4 ± 1.7
β-Elementene	19.44	3 ± 0.9	1.9 ± 0.53	1.2 ± 0.22		2.7 ± 0.75	2.3 ± 0.3
Methyl(E)-cinnamate	20.45	1 ± 1.1	1.8 ± 1	0.6 ± 0.69	1 ± 0.32	0.8 ± 1.3	0.6 ± 0.08
β-Caryophyllene	20.86	3 ± 0.41	2.4 ± 0.79	1.1 ± 0.22	0.6 ± 0.19	2.8 ± 0.52	3.1 ± 0.53
γ-Elementene	21.26	28 ± 0.57	2.6 ± 0.51	1.9 ± 0.34	0.6 ± 0.3	2.8 ± 0.54	2.4 ± 0.29
α-Humulene	22.04	1.2 ± 0.18	0.9 ± 0.25	0.5 ± 0.1	0.3 ± 0.15	1 ± 0.25	1.3 ± 0.39
β-Cubebene	23.2	1.3 ± 0.24	0.9 ± 0.4	0.7 ± 0.06	0.8 ± 0.18	0.9 ± 0.26	7.1 ± 0.57
β-Bisabolene	24.58				1.2 ± 0.31		
α-Farnesene	25.74	2 ± 0.51	1.7 ± 0.6	0.9 ± 0.33	0.1 ± 0.15	2.1 ± 0.21	2.5 ± 0.33
Spathulenol	27.6	15 ± 1.9	13.4 ± 1	2.7 ± 0.5	0.7 ± 0.26	14.3 ± 0.4	11.3 ± 0.9

^a Listed in order of retention time; ^b RT = retention time (min).

^c ot53 = 'Lemon' (Richters); ot58 = 'Lemon' (Companion); ot61 = 'Lemon Mrs. Burns'; ot62 = 'New Guinea'; ot66 = 'Lemon' (Nichols); ot70 = 'Sweet Dani'.

^d formerly *O. basilicum*, included here for its citral composition. ^e Values reported as an average of three replicates + SD.

^f Retention times for neral and geranial, respectively.

Table 5. Chemical composition of *Ocimum minimum* and *O. kilimandscharicum* accessions, grown under field conditions (Purdue University)

Volatile oil constituents ^a	RT ^b	<i>Minimum</i>			<i>Kilimandscharicum</i>	
		ot34 ^c	ot35	ot60	ot3	ot50
(Relative percentage of total volatile oil) ^d						
α-Pinene	4.79	0.4 ± 0.17	1.9 ± 0.26	0.8 ± 0.32		
β-Pinene	4.96	0.5 ± 0.33	0.6 ± 0.06	0.2 ± 0.26	0.8 ± 0.27	
1,8-Cineole	6.08	6.1 ± 2	16.3 ± 1.9	1.3 ± 0.5	4.4 ± 1.9	10.2 ± 1.7
γ-Terpineol	7.31	2.1 ± 0.27	1.4 ± 0.35	1 ± 0.82		
Linalool	8.1	46.4 ± 4.1	46.6 ± 2.6	66.9 ± 16.3	1.2 ± 0.2	53.1 ± 2
Terpinolene	8.12				0.9 ± 0.3	0.8 ± 0.4
Camphor	9.24	6.4 ± 1	0.6 ± 0.19	1.1 ± 0.53	78.3 ± 5.1	19.3 ± 0.53
Terpin-4-ol	10.95	5.1 ± 2.77	3.1 ± 0.29		0.9 ± 0.2	0.4 ± 0
Methylchavicol	11.72	2 ± 0.29	1.7 ± 0.17	0.7 ± 0.37	2.1 ± 0.22	0.9 ± 0.06
Eugenol	18.72	1.8 ± 0.44	2.9 ± 0.29	0.5 ± 0.37	1.8 ± 0.5	1.2 ± 0.1
β-Elementene	19.44	1.5 ± 1.37	0.4 ± 0.4	8.1 ± 13.9		1.9 ± 0.31
Methyleugenol	20.03	3 ± 1	5.4 ± 0.7	2.4 ± 1	0.4 ± 0.6	0.7 ± 0.2
α-Humulene	22.04	1.2 ± 1	0.5 ± 0.05	1.1 ± 0.77		
β-Cubebene	23.28	1 ± 0.52	2.1 ± 0.46	2.2 ± 0.8	0.9 ± 0.28	1.2 ± 0.15
allo-Aromandrene	24.28	2.4 ± 0.64	1.9 ± 0.21	1.3 ± 0.1		
β-Bisabolene	24.58	1.6 ± 0.61	2 ± 0.81	1.7 ± 0.52		
Spathulenol	27.6	1.7 ± 0.59	1.1 ± 0.25	1.4 ± 0.49	0.7 ± 0.2	1.9 ± 0.3

^a Listed in order of retention time; ^b RT = retention time (min).

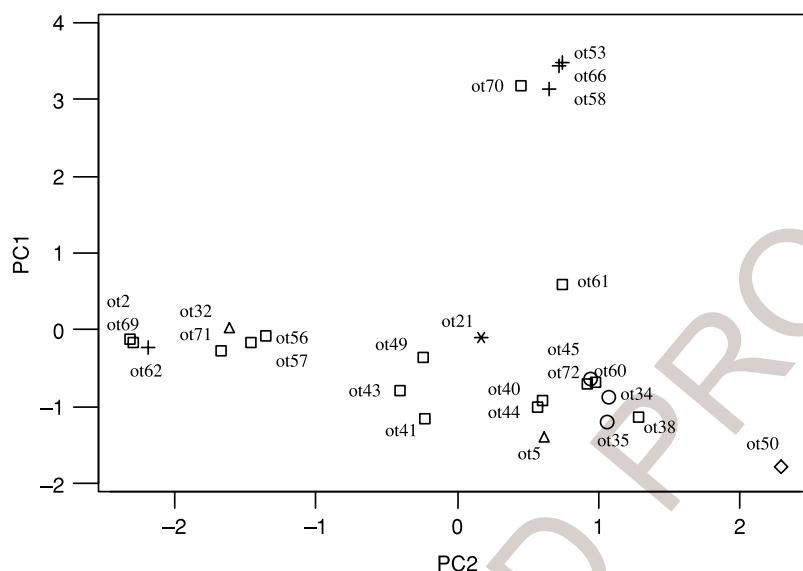
^c ot34 = 'Bush basil'; ot35 = 'Spicy basil'; ot60 = 'Dwarf Opal'; ot3 = *O. kilimandscharicum* (Kew); ot50 = 'African Blue'.

^d Values reported as an average of three replicates + SD.

camphor (6.3%), an important secondary compound in 'Bush' (ot34), 1,8-cineole (16.3%) in 'Spice Globe' (ot35) and β-elementene (8.1%) in 'Dwarf Opal' (ot60). Two accessions of *O. kilimandscharicum* were observed, the first (ot3) a camphor-rich (78%) accession, and the second (ot50) a hybrid between *O. basilicum* and *O. kilimandscharicum* rich in linalool (53%) and camphor (19%).

A comparative analysis of the 27 identified compounds by PCA allowed us to select the best distinctions based upon seven major compounds. The PCA allowed us to examine the correlation of each compound, and the contribution of each compound (eigenvalue) in the discrimination of each accession (Fig. 1).

The first three principal components (PC) corresponded to 74% of the total variation (Table 6). The spatial



1 Figure 1. Principal component analysis of seven volatile oil constituents in 26 *Ocimum* accessions. (□) *Ocimum basilicum*; (*) *O. americanum* var. *pilosum*; (□) *O. americanum* var. *americanum*; (+) *O. citriodorum*; (○) *O. minimum*; (□) *O. basilicum* × *O. kilimandscharicum*

Table 6. Eigenvalues and loadings on principal component analysis of variation of volatile oil constituents in *Ocimum* accessions

	Principal axes		
	I	II	III
Eigenvalue	2.34	1.61	1.23
Proportion (%)	33	23	18
Cumulative (%)	33	56	74
Loadings			
1,8-Cineole	-0.346	0.272	0.111
Linalool	-0.282	0.545	-0.059
Camphor	-0.195	0.270	0.244
Methylchavicol	-0.079	-0.629	0.513
Citral	0.620	0.195	0.069
Methyl(<i>E</i>)-cinnamate	-0.004	-0.278	-0.804
Spathulenol	0.610	0.212	0.104

relationship of the 26 accessions using the first three vectors from this analysis is illustrated in Fig. 1. The first component explains 33.4% of the variation and has an eigenvalue of 2.3, which consists of citral and spathulenol as major constituents (Table 6). Citral and spathulenol showed a correlation value of 94% (data not shown). The PC2 explains 23% of the variation, with an eigenvalue of 1.6. The PC2 is bipolar with linalool contrasting with methylchavicol. Both compounds showed a negative correlation of 47%. The PC3 accounts for 17.5% of the variation, and has an eigenvalue of 1.2, with methyl(*E*)-cinnamate as the major component of this vector. The PC vectors were able to separate the accessions in five groups, which do not consist of five groups of species studied, based on the major volatile oils constituents: (1) citral–spathulenol-rich accessions; (2) linalool-rich

accessions; (3) methylchavicol accessions; (4) linalool–methylchavicol accessions; and (5) methyl(*E*)-cinnamate accessions (Fig. 1).

The use of secondary metabolites in plant taxonomy is well recognized,^{27,28} and often can provide useful characteristics for taxonomic classification. Multivariate analysis techniques have been used successfully in the study of infraspecific^{18,29} and interspecific variability of essential oils.^{30,31}

Grayer *et al.*²³ reported that the use of one single volatile oil to describe a chemotype is problematic, since there are often two or more major compounds, which might be present in nearly equal amounts and many minor compounds. A system of chemical descriptors based on the combination of major compounds (those with higher than 20%) rather than the sole dominant compounds was instead proposed by Grayer *et al.*²³

The large genetic variation within *Ocimum* species continues to show a segregation of several minor compounds, including many of the sesquiterpenes, making the use of essential oils that are difficult to establish as a reliable general descriptor for all accessions and varieties. Although chemotypes have been found mainly among the monoterpenes, sesquiterpenes have been shown to play a significant role in the establishment of chemotypes in other species such as *Thymus* species.¹⁸

Chemotaxonomic conclusions using solely volatile oils profiles need to be tempered when addressing both a partially domesticated and highly polymorphic group such as *Ocimum*. It is noteworthy that most studies on chemosystematics using essential oils are made on natural populations,²⁹ while many of the basil, particularly the *O. basilicum*s, have been subject to some form

of domestication over a long period.⁴ The use of essential oils as a taxonomic descriptor coupled with genetic fingerprinting^{14,15} would provide in basil a potent combination for taxonomic classifications of this genus.

The groups of *Ocimum* species based on morphological characteristics do not correspond to the groups based on volatile oils constituents. Each group of volatile oil profiles can occur in different species, for example, the citral/spathulenol type occurs in both *O. × citriodorum* and *O. basilicum*; linalool-rich types are frequent in *O. basilicum* varieties, *O. minimum*, *O. kilimandscharicum* and *O. × citriodorum*. The presence of methyl-cinnamate-rich types in both *O. americanum* and *O. basilicum* clearly shows that species and essential oil profiles do not necessarily correspond. Basil cultivars are the result of many years of breeding and selection. As morphological traits and chemical characteristic segregation are not necessarily linked, plants can obtain morphological characteristics from one parent or chemical characteristics from the other. Therefore, several variations can be found at infraspecific level.

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