Essential oil composition of three sweet basil (*Ocimum basilicum* L.) cultivars

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S u m m a r y

In the studies conducted in 2010–2011, the chemical composition of the essential oils of three sweet basil (*Ocimum basilicum* L.) cultivars were investigated by GC-MS method.
Forty eight compounds (95.08% of the total oil) were identified in the essential oil of *O. basilicum* L. *cv.* 'Thai Siam'. The main components found in the oil were linalool (24.60 and 36.60% in 2010 and 2011, respectively), (E)-methyl cinnamate (18.73 and 21.90%) and methyl chavicol (5.57 and 7.50%). In the essential oil of *O. basilicum* L. *cv.* 'Bolloso Napoletano', forty constituents were identified, representing 97% of total oil. Linalool (41.09 and 47.75%), methyl chavicol (14.34 and 20.21%) and 1,8-cineole (7.21 and 10.23%) were the major components. Similarly, linalool (37.51 and 48.65%), methyl chavicol (13.41 and 18.55%) and 1,8-cineole (7.72 and 12.59%) were found to be the main constituents of the oil of *O. basilicum* L. *cv.* 'A Foglie di Lattuga'. A total of 35 compounds were identified in this oil, representing 95.24% of the total oil content.

Key words: *Ocimum basilicum, steam distillation, essential oil, linalool, methyl chavicol, methyl cinnamate*

INTRODUCTION

Sweet basil (*Ocimum basilicum* L.), the oldest spices belonging to the *Lamiaceae* family, is commercially and extensively cultivated for essential oil production in many continents around the world [1]. Oil and oleoresin of basil are widely utilized for flavor and fragrance in the food, pharmaceutical, cosmetic, and aromatherapy industries [2]. A number of authors have mentioned the antimicrobial, antifungal and insect-repelling properties of basil oil. Several components, such as camphor and 1,8-cineole have been suggested as agents in allelopathy reactions [3-8]. In traditional medicine the oil is used to relieve respiratory problems, against intestinal parasites and as a first aid treatment for wasp stings [9]. Even the smell of basil is considered to be curative [10].

The chemical composition of *O. basilicum* L. essential oil depends on chemotype, leaf and flower colors, aroma and origin of plants [11-12]. Lawrence [13] classified four major chemotypes of basil based on essential oil composition: 1) methyl chavicol (estragole)-rich, 2) linalool-rich, 3) methyl eugenol-rich, 4) methyl cinnamate-rich, and also numerous subtypes.

Basil cultivars also vary significantly in their essential oil composition. According to their geographical origin and on the basis of their major constituents, they are classified in four chemotypes [14]: 1) European chemotype: the oil is characterized by high amounts of linalool (35–50%) and estragole (15–25%); 2) Reunion chemotype (estragole basil): its main essential oil component is estragole (80% or more); 3) tropical chemotype (cinnamon basil): oil is dominated by methyl cinnamate and 4) eugenol chemotype with major oil component eugenol.

The wide distribution throughout the world, the availability of various chemotypes and the production of new cultivars have resulted in a great variation in the essential oil composition and aroma among basil species [15]. The oils from different types of basil or different production areas are of different distinct flavors and different value on the world market [16-17]. Scented basils (cinnamon, licorice and lemon) are used in potpourri, jellies, honeys, vinegars and baked goods [18]. In the perfumery the chemical composition of basil oil is also of a great importance. The oil (with linalool and methyl chavicol as the main components), is often considered to have the finest flavor and the highest quality [19].

The main objective of this study was to chemically characterize the essential oils of three sweet basil cultivar cultivated in North-Western Poland in 2010–2011.

MATERIAL AND METHODS

Plant

In 2010–2011, the field experiment was conducted at the Horticultural Experimental Station in Dołuje near Szczecin. The following sweet basil cultivars were analysed: ‘Thai Siam’, ‘Bolloso Napoletano’ and ‘A Foglie di Lattuga’.

The basil seeds used in the experiment came from commercial seed company World Sementi Polish Distribution in Szczecin. The experiment was established in a system of random blocks, in four replications. In both years of the experiment the seeds were sown directly into the open field on 23 May with 40 cm row spacing. The area of one plot was 1.44 m^2 (1.2 x 1.2 m^2), and of the whole experiment – 30 m^2. For 1.44 m^2 of the field, 0.86 g of seeds were used. During vegetation manual weeding and soil loosening were performed.

Aerial parts of basil were collected at the beginning of flowering ('Thai Siam' on 16 August 2010 and 2 September 2011; 'Bolloso Napoletano' and 'A Foglie di Lattuga' on 11 August 2010 and 28 July 2011). The plants were cut at 10-15 cm above the ground. For the laboratory analyses 100 g samples, from each experimental plot (4 x 100 g), were taken. Subsequently, the samples of each cultivar were combined, mixed and dried in a shady and well ventilated place at a room temperature. The dried basil herb was rubbed through the sieve of mesh diameter of 4–5 mm and stored in paper bags. Representative samples (100 g) were used for essential oil isolation.

Isolation of the essential oil

Steam distillation

Steam distillation was carried out by passing steam into a one-liter round-bottomed flask containing the dried plant material (10 g) and 400 ml of distilled water for 90 min and collecting the condensate (water and oil) in a Erlenmeyer flask. This method of obtaining essential oil is recommended by Charles and Simon [20]. The condensate was saturated with sodium chloride and extracted three times with methylene chloride (100 ml) to extract the essential oil completely. Sodium sulphate was added to the methylene chloride in order to remove moisture. Subsequently, the solvent was removed by rotary evaporation at 40°C.

Extractions were performed at least three times, and the mean values were reported. The essential oil content was calculated based on dried weight of plant materials.

The GC-MS analysis

The GC-MS analysis was carried out with a Hewlett-Packard 6890 gas chromatograph fitted with a fused silica HP-5MS capillary column (30 m x 0.25 mm; film thickness 0.25 μm). Helium (at 2 ml/min) was used as a carrier gas. Samples (2 μl) as solutions in dichloromethane were injected in the split mode at a ratio of 5.4:1. The injector and the transfer line were kept at 280°C.

The column was maintained at 40°C for 5 min, then increased to 230°C at a rate of 60°C/min (kept constant for 10 min), and then increased to a final temperature of 280°C at a rate of 30°C/min (kept constant for 30 min). The gas chromatograph was coupled to a Hewlett-Packard 5973 mass selective detector. The MS operating parameters were as follows: ionization voltage 70 eV; ion source temperature 230°C. Data were collected at a mass range of m/z 40–800.

Compound identification

The constituents of the essential oils were identified by calculation of their retention indices under temperature-programmed conditions for n-alkanes (C_6–C_{24}) and the oil on a HP-5MS column under the same chromatographic conditions. Identification of individual compounds was based on the NIST/EPA/NIH Mass Spectral Library (2002 version); the library NBS75K in the Enhanced ChemStation G1701AA or with authentic compounds (eugenol, linalool, (+)-camphor, (E)-methyl cinnamate) and confirmed by comparison of their retention indices with authentic compounds or with those of reported in the literature [12, 21].

The relative percentage of the oil constituents was calculated from GC peak areas.

Chemicals

Dichloromethane (pure p.a.) was purchased from Chempur and used after distillation.

(E)-Methyl cinnamate purum, ≥99.0% (GC), eugenol (99%), linalool (97%) and (+)-camphor (98%) were purchased from Sigma-Aldrich.

Statistical analysis

Several results of the study (tab. 1 and 3) were subjected to the analysis of variance which was performed with AWAR programme, made by Department of Applied Informatics, Institute of Soil Science and Plant Cultivation, Puławy, Poland. The means were separated by the Tukey's test at $p=0.05$.

Table 1.

Essential oil content in *O. basilicum* cultivars in 2010–2011

Cultivar	Oil content (%)		
	2010	2011	mean
Thai Siam	0.60	0.50	0.55
Bolloso Napoletano	0.45	0.35	0.40
A Foglie di Lattuga	0.35	0.40	0.38
mean	0.47	0.42	0.44
$LSD_{0.05}$ cultivar		n.s.	
year		n.s.	
interaction		n.s.	

Table 2.

Percentage composition of the essential oils of three *O. basilicum* cultivars in 2010–2011

Components	Cultivar						
		A		B		C	
	RI	2010	2011	2010	2011	2010	2011
β-Pinene	969	-	-	0.08	0.10	-	-
1-Octen-3-ol	972	0.11	0.29	-	-	-	0.08
β-Myrcene	976	-	-	0.08	0.26	-	-
1,8-Cineole	**998**	**2.39**	**2.97**	**10.23**	**7.21**	**12.59**	**7.72**
cis-β-Terpineol	1014	-	-	0.15	0.09	0.18	0.16
cis-Linalool oxide	1016	0.76	0.21	0.95	0.57	0.67	0.45
trans-Linalool oxide	1022	0.65	0.42	0.89	0.62	0.70	0.47
Linalool	**1030**	**24.60**	**36.60**	**41.09**	**47.75**	**37.51**	**48.65**
Phenylethyl alcohol	1034	-	0.34	-	-	-	-
4-Acetyl-1-methyl-cyclohexene	1040	0.17	0.40	0.30	0.65	0.28	1.14
(+)-Camphor	1161	0.65	0.61	1.02	0.77	0.75	0.84
Lilac aldehyde C	1165	0.08	0.30	-	-	-	-
Borneol	1179	0.34	0.43	-	-	-	-
1-Terpinen-4-ol	1189	2.04	1.09	0.26	0.23	1.95	0.26
α-Terpineol	1200	0.90	0.80	0.91	0.71	1.00	0.77
Methyl chavicol	**1205**	**7.50**	**5.57**	**14.34**	**20.21**	**13.41**	**18.55**
Isoestragole	1216	0.40	0.42	-	-	-	-
Fenchyl acetate	1221	0.15	0.18	-	-	-	-
Exo-2-hydroxy-Cineole	1224	0.09	0.44	-	-	-	-
Nerol	1246	0.24	0.23	-	-	-	-
Chavicol	1252	-	1.99	-	-	-	-
(-)-Bornyl acetate	1268	0.25	0.85	0.57	0.81	0.40	0.62
(Z)-Methyl cinnamate	1284	5.53	2.66	-	-	-	-
Exo-2-hydroxy-cineole acetate	1302	-	-	0.07	0.07	-	-
α-Cubebene	1309	0.12	0.11	0.11	0.10	0.09	0.08
Eugenol	1316	2.41	2.84	4.81	2.39	1.60	2.33
(-)-β-Elemene	1326	0.19	0.31	0.59	0.39	0.52	0.37
(E)-Methyl cinnamate	**1363**	**21.90**	**18.73**	-	-	-	-
Methyl eugenol	1373	0.60	-	0.67	0.33	-	-
β-Caryophyllene	1403	0.23	0.39	0.23	0.10	0.29	-
α-Bergamotene	1418	1.17	0.72	2.31	1.60	6.51	1.10

Components	Cultivar						
		A		B		C	
	RI	2010	2011	2010	2011	2010	2011
α-Guaiene	1424	0.44	0.35	0.58	0.41	0.73	0.32
(Z)-β-Farnesene	1440	0.67	0.52	0.45	0.42	0.37	0.21
α-Humulene	1448	0.40	0.32	0.60	0.43	0.51	0.34
(+)-Epi-bicyclo-sesquiphellandrene	1450	0.56	0.33	0.41	0.33	0.41	0.27
γ-Muurolene	1474	0.22	0.18	-	-	-	-
β-Cubebene	1483	1.16	1.05	0.99	0.96	1.52	0.90
β-Selinene	1491	0.42	0.18	0.16	0.13	-	-
Bicyclogermacrene	1502	0.90	0.71	0.39	0.40	0.33	0.36
α-Bulnesene	1512	0.84	0.44	0.52	0.43	0.49	0.38
γ-Cadinene	1523	2.22	1.11	1.68	1.23	1.83	1.23
Calamenene	1531	0.53	0.31	0.52	0.38	0.73	0.33
Ledol	1557	-	-	-	-	0.21	-
(+)-Nerolidol	1574	0.61	0.40	-	-	-	-
(-)-Spathulenol	1597	0.87	0.32	0.82	0.37	0.60	0.38
Caryophyllene oxide	1605	0.28	0.18	0.19	0.07	0.15	0.24
Virdiflorol	1614	0.26	0.28	1.15	1.27	0.15	0.26
Cadine-1,4-diene	1633	-	-	0.90	0.71	0.13	0.44
Cubenol	1636	1.30	0.89	-	-	0.93	0.83
(-)-β-Cadinene	**1663**	**6.96**	**5.78**	**5.67**	**3.90**	**6.33**	**4.59**
α-Cadinol	1677	1.10	1.21	1.05	0.90	1.15	1.07
Spathulenol	1681	0.18	-	0.25	-	-	-
α-Bisabolol	1704	0.49	0.22	0.42	0.16	0.06	-
Tetradecanal	1723	0.11	-	0.11	-	-	-
7,10,13-Hexadeca-trienal	1874	0.27	-	-	-	-	-
Phytol	2025	0.82	0.32	0.48	0.13	0.16	0.21
Total identified		***95.08***	***95.00***	***97.00***	***97.59***	***95.24***	***95.95***
Monoterpene hydrocarbons		-	-	0.16	0.36	-	-
Oxygenated monoterpenes		33.06	44.83	56.14	58.83	55.75	59.94
Sesquiterpene hydrocarbons		17.03	12.81	16.11	11.92	20.79	10.92

Components	Cultivar						
		A		B		C	
	RI	2010	2011	2010	2011	2010	2011
Oxygenated sesquiterpenes		5.09	3.50	3.88	2.77	3.25	2.78
Aromatic compounds		38.34	32.55	19.82	22.93	15.01	20.88
Others		1.56	1.31	0.89	0.78	0.44	1.43

Components are listed in order of elution on HP-5MS column
A-'Thai Siam'
B-'Bolloso Napoletano'
C-'A Foglie di Lattuga'

Table 3.

Statistical analysis of the content of main constituents of essential oils of three basil cultivars (mean values from 2010–2011)

Essential oil constituent (*factor I*)	Cultivar (*factor II*)			
	A	**B**	**C**	**Mean**
1.8-Cineole	2.68 de	8.72 c*	10.16 c	7.19 bc
cis-Linalool oxide	0.49 e	0.76 f	0.56 f	0.60 c
trans-Linalool oxide	0.54 e	0.76 f	0.59 f	0.63 c
Linalool	30.60 a	44.42 a	43.08 a	39.37 a
(+)-Camphor	0.63 e	0.90 ef	0.80 f	0.77 c
1-Terpinen-4-ol	1.57 de	0.25 f	1.11 ef	0.97 c
α-Terpineol	0.85 e	0.81 f	0.89 f	0.85 c
Methyl chavicol	6.54 c	17.28 b	15.98 b	13.26 b
(Z)-Methyl cinnamate	4.10 cd	-	-	1.37 c
Eugenol	2.63 de	3.60 de	1.97 ef	2.73 c
(E)-Methyl cinnamate	20.32 b	-	-	6.77 bc
α-Bergamotene	0.95 e	1.96 ef	3.81 de	2.24 c
(Z)-β-Farnesene	0.60 e	0.44 f	0.29 f	0.44 c
β-Cubebene	1.11 e	0.98 ef	1.21 ef	1.10 c
Bicyclogermacrene	0.81 e	0.40 f	0.35 f	0.52 c
α-Bulnesene	0.64 e	0.48 f	0.44 f	0.52 c

Essential oil constituent (*factor I*)	Cultivar (*factor II*)			
	A	**B**	**C**	**Mean**
γ-Cadinene	1.67 de	1.46 ef	1.53 ef	1.55 c
Virdiflorol	0.27 e	1.21 ef	0.21 f	0.56 c
(-)-β-Cadinene	6.37 c	4.79 d	5.46 d	5.54 c
α-Cadinol	1.16 e	0.98 ef	1.11 ef	1.08 c
Mean	**4.22**	**4.51**	**4.48**	**4.40**
$LSD_{\alpha=0.05}$ for factor I	7.369			
$LSD_{\alpha=0.05}$ for factor II	n.s.			
$LSD_{\alpha=0.05}$ for interaction I x II	2.788			

*Means followed by the same letters are not significantly different for $p < 0.05$

A- ‘Thai Siam’
B- ‘Bolloso Napoletano’
C- ‘A Foglie di Lattuga’

RESULTS

The essential oil content obtained from aerial parts of selected basil cultivars are shown in table 1.

The presented data indicate that the cultivar, as well as the year of cultivation, had no significant effect on the content of essential oil which average amount was 0.44%.

Considering three oils together, a total of fifty six different compounds were identified: 48 for ‘Thai Siam’ cultivar (95.08% of the total oil), 40 for ‘Bolloso Napoletano’ cultivar (97.00% of the oil) and 35 for ‘A Foglie di Lattuga’ cultivar (95.24% of the oil).

The main constituents found in the oil of ‘Thai Siam’ (sample A) were linalool (24.60 and 36.60%), (E)-methyl cinnamate (18.73 and 21.90%), methyl chavicol (5.57 and 7.50%) and (-)-β-cadinene (5.78 and 6.96%). In the oil of ‘Bolloso Napoletano’ (sample B) linalool (41.09 and 47.75%), methyl chavicol (14.34 and 20.21%), 1,8-cineole (7.21 and 10.23%) and (-)-β-cadinene (3.90 and 5.67%) were the major components. The volatile oil of ‘A Foglie di Lattuga’ (sample C) was rich in linalool (37.51 and 48.65%), methyl chavicol (13.41 and 18.55%), 1,8-cineole (7.72 and 12.595) and (-)-β-cadinene (4.59 and 6.33%).

Oxygenated monoterpenes (33.06–59.94%), aromatic compounds (15.01–38.34%) and sesquiterpene hydrocarbons (10.92–20.79%) dominated in the studied oils. Moreover, the two studied cultivars of *O. basilicum* ('Bolloso Napoletano' and 'A Foglie di Lattuga') have a similar content of oxygenated monoterpenes and aromatic compounds and similar oil composition. In cultivar 'Thai Siam', oxygenated monoterpenes and aromatic compounds were present in quite similar amounts, 33.06–44.83% and 32.55–38.34%, respectively. Aromatic compounds such as, (E)-methyl cinnamate and (Z)-methyl cinnamate were present only in this oil (tab. 2).

Based on the statistical analysis of the two-year study results it was proved that the influence of essential oil constituent and interaction between both experimental factors (essential oil constituent and cultivar) on the content of main essential oil constituents of three basil cultivars was statistically significant (tab. 3).

It was also proved that the basil cultivar had no significant influence on the content of analyzed oil constituents.

Among 20 comparable oil constituents, regardless from cultivar, significantly higher content was noted for linalool (39.37%), while significantly lower for methyl chavicol (13.26%), 1,8-cineole (7.19%) and (E)-methyl cinnamate (6.77%). Moreover, there were no statistically significant differences between the contents of 1,8-cineole and (E)-methyl cinnamate and other oil constituents which amounts ranged from 0.44 to 5.54%.

Measuring significance of the interaction between both experimental factors, it was concluded that 'Thai Siam' cultivar was characterized by the highest contents of linalool and (E)-methyl cinnamate, whereas 'Bolloso Napoletano' and 'A Foglie di Lattuga' by the highest amounts of linalool, methyl chavicol and 1,8-cineole.

DISCUSSION

The oil content of sweet basil in this study (0.38–0.55%) was similar to several literature reports [9, 22-25]. Suchorska and Osińska studied five forms of sweet basils from Germany, Romains, Hungary and Egypt and reported that the oil content varied from 0.1 to 0.55% [22]. A study by Marotti et al. [9] showed that the content of essential oil in herb of 10 Italian basil cultivars ranged from 0.3 to 0.8%. Galambosi and Szebeni [23] reported oil contents in basil herb from 0.38 to 1.29%, while Seidler-Łożykowska [24] from 0.23 to 1.67%. In a large study on 270 sweet basil accession in Germany, oil content varied from traces to 2.65% [25]. Such variations in the essential oil content of basil across countries might be attributed to the varied agroclimatic conditions of the regions.

The chemical composition of basil oil has been the subject of considerable studies. In the oils obtained from aerial parts of basil grown in Colombia and Bulgaria, linalool and methyl cinnamate were reported as major components of essential oils, respectively [8, 26]. In basil cultivars from Australia, methyl chavicol, linalool, methyl cinnamate, a mixture of linalool/methyl cinnamate, and linalool/methyl chacivol were reported as the main components [27]. Sweet basil oil distilled in France, Italy, Bulgaria, Egypt, Hungary, South Africa and occasionally in the USA, contains almost identical amounts of methyl chavicol (20–43%) and linalool (37–55%) [28].

The dominant constituent in all oil samples was linalool, ranging from 24.60 to 48.65% of total oils. This indicates that the investigated cultivars could be considered as originating from the European chemotype. Essential oil from 'Thai Siam' had similar chemical composition to 'Cinnamon' studied by Nurzyńska-Wierdak [29], however, it contained less (E)-methyl cinnamate. 'Bolloso Napoletano' and 'A Foglie di Lattuga' had similar essential oil composition, although the first exhibited higher eugenol content and the second higher (-)-β-cadinene content. Due to the high content of linalool, methyl chavicol and methyl cinnamte, the studied cultivars may become applied in food and perfume industries.

CONCLUSIONS

1. The main compounds of three sweet basil cultivars oils were linalool and methyl chavicol.
2. 'Bolloso Napoletano' and 'A Foglie di Lattuga' had similar essential oil composition and belong to "linalool and methyl chavicol" chemotype.
3. (E)-methyl cinnamate and (Z)-methyl cinnamate were found only in 'Thai Siam' essential oil.
4. 'Thai Siam' cultivar belong to "linalool and methyl cinnamate" chemotype.

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SKŁAD OLEJKU ETERYCZNEGO Z TRZECH ODMIAN BAZYLII POSPOLITEJ (*OCIMUM BASILICUM* L.)

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S t r e s z c z e n i e

W latach 2010–2011 badano skład chemiczny olejków eterycznych z trzech odmian bazylii pospolitej (*Ocimum basilicum* L.): 'Thai Siam', 'Bolloso Napoletano' i 'A Foglie di Lattuga'. W olejku eterycznym 'Thai Siam' zidentyfikowano 48 związków. Linalol (24,60 i 36,60%, odpowiednio w roku 2010 i 2011), (E)-cynamonian metylu (18,73 i 21,90%) i metylochawikol (5,57 i 7,50%) stanowiły jego główne składniki. Linalol (41,09 i 47,75%), metylochawikol (14,34 i 20,21%) i 1,8-cineol (7,21 i 10,23%) stanowiły dominujące składniki olejku 'Bolloso Napoletano', w którym zidentyfikowano łącznie 40 związków. Podobnie linalol (37,51 i 48,65%), metylochawikol (13,41 i 18,55%) i 1,8-cineol (7,72 i 12,59%), stanowiły główne składniki olejku 'A Foglie di Lattuga'. W olejku tym zidentyfikowano 35 związków. W badanych olejkach bazyliowych zidentyfikowano od 95 do 97% związków.

Słowa kluczowe: *Ocimum basilicum, destylacja z parą wodną, olejek eteryczny, linalol, metylochawikol, cynamonian metylu*