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SOURCES OF VARIABILITY IN ESSENTIAL OIL COMPOSITION OF *OCIMUM AMERICANUM* AND *OCIMUM TENUIFLORUM*

B. Bernhardt*, K. Szabó and J. Bernáth

Department of Medicinal and Aromatic Plants, Faculty of Horticultural Science, Corvinus University of Budapest, H-1118 Budapest, Villányi út 29–43. Hungary

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Basil has traditionally been used for a long time in medicine and gastronomy. Essential oil is the most important active substance of the drug, which influences the aroma and the effect of the plant. Although the compositions of essential oils vary in different basil cultivars, the main components are oxygenated monoterpenes and phenylpropane derivates. The high chemical variation is most likely caused by interspecific hybridization. Various factors, like genetic background, ontogenesis, morphogenesis, abiotic factors, essential oil extraction method, drying, and storage, are responsible for the variant essential oil composition.

Keywords: basil, essential oil, monoterpenes, sesquiterpenes, phenylpropane derivates, ontogenesis, morphogenesis, environmental factor, extraction, drying, storage

Common basil (*Ocimum basilicum* L.) is the most economically important species amongst *Ocimum* spp. Aromatic leaves, flowering tops, and essential oils are used in food industry to flavour foods and beverages, in fragrance ingredients (perfumes, soaps, hair dressings, dental creams, and mouth washes), and in traditional medicine (Holm, 1999; De Masi et al., 2006). In addition, *Ocimum americanum, Ocimum* × *citriodorum, Ocimum gratissimum, Ocimum selloi* and *Ocimum tenuiflorum* also have an important role in the food industry; their essential oils are often used, too. As medicine, it has antibacterial, antifungal, insecticidal, and hepatoprotective activity. Also it has antiulcerogenic effect and relaxes the smooth muscle. It has antioxidative effect and contains antimicrobial substances (Holm, 1999). The main active agent is its essential oil, which accumulates in the organs in different quantity. However, several biotic and abiotic factors affect the level of accumulation and its components. Genetic variability, ontogenesis, morphogenesis, essential oil extraction method, drying, and storage also influence the chemical composition of basil.

1. Composition of the essential oil in *Ocimum americanum* and *Ocimum tenuiflorum*

Several chemotaxa exist in the genus *Ocimum,* which vary in their essential oil composition. This difference should be mainly due to polymorphism, which is caused by interspecific hybridization (Hasegawa et al., 1997). Although essential oil compositions vary in different basil cultivars, the main components are oxygenated monoterpenes and phenylpropane derivates as mentioned by Hiltunen and Holm (1999). Chemotype classification based on

* To whom correspondence should be addressed.
Phone: +36-1-482-6250; fax: +36-1-482-6330; e-mail: botond.bernhardt@uni-corvinus.hu

just one main component of essential oil is problematic, because frequently one plant contains two or more compounds in nearly equal amounts. To identify the oil profile a threshold level (e.g. 20% of total essential oil content) must be fixed (LABRA et al., 2004). According to the literature, (see in the Tables 1–2) we tried to summarize the chemical characterization of *Ocimum americanum* and *Ocimum tenuiflorum* as follows:

Ocimum americanum **(syn.** *O. canum***)**
a) **Chemovarietas** - *trans*-β-ocimene
aa) **chemoform** - *trans*-β-ocimene - 1,8-cineole
ab) **chemoform** - *trans*-β-ocimene - methyl cinnamate
b) **Chemovarietas** - camphor
c) **Chemovarietas**- 1,8-cineole
ca) **chemoform**- 1,8-cineole - *trans*-β-ocimene
cb) **chemoform** - 1,8-cineole - linalool - methyl cinnamate
d) **Chemovarietas** - citral
da) **chemoform** - citral - linalool
e) **Chemovarietas** - linalool
f) **Chemovarietas** - terpinen-4-ol
fa) **chemoform** - terpinen-4-ol - linalool
g) **Chemovarietas**- methyl cinnamate
ga) **chemoform** - methyl cinnamate - *trans*-β-ocimene
gb) **chemoform** - methyl cinnamate - 1,8-cineole - linalool
h) **Chemovarietas** - eugenol

O. tenuiflorum **(syn.** *O. sanctum***)**
a) **Chemovarietas**- 1,8-cineole
aa) **chemoform** - 1,8-cineole - β-bisabolene - methyl chavicol - eugenol
b) **Chemovarietas**- β-bisabolene
ba) **chemoform** - β-bisabolene - 1,8-cineole - methyl chavicol - eugenol
c) **Chemovarietas** - β-caryophyllene
ca) **chemoform** - β-caryophyllene - β-elemene - methyl eugenol - eugenol
cb) **chemoform** - β-caryophyllene - β-elemene - methyl eugenol
cc) **chemoform** - β-caryophyllene - β-elemene - eugenol
cd) **chemoform** - β-caryophyllene - methyl eugenol
d) **Chemovarietas** - β-elemene
da) **chemoform** - β-elemene - β-caryophyllene - methyl eugenol - eugenol
db) **chemoform** - β-elemene - β-caryophyllene - methyl eugenol
dc) **chemoform** - β-elemene - β-caryophyllene - eugenol
dd) **chemoform** - β-elemene - methyl eugenol
e) **Chemovarietas** - methyl chavicol
ea) **chemoform** - methyl chavicol - 1,8-cineole - β-bisabolene -eugenol
eb) **chemoform** - methyl chavicol – eugenol
f) **Chemovarietas** - methyl eugenol
fa) **chemoform** - methyl eugenol - β-caryophyllene - β-elemene- eugenol
fb) **chemoform** - methyl eugenol - β-caryophyllene- β-elemene
fc) **chemoform** - methyl eugenol - β-elemene
fd) **chemoform** - methyl eugenol - β-caryophyllene

g) **Chemovarietas** - eugenol
ga) **chemoform** - eugenol - 1,8-cineole - β-bisabolene -methyl chavicol
gb) **chemoform** - eugenol - β-caryophyllene - β-elemene- methyl eugenol
gc) **chemoform** - eugenol - methyl chavicol
gd) **chemoform** - eugenol- β-caryophyllene - β-elemene

In food industry, chemotaxons with few or no methyl chavicol content are the most valuable ones.

2. Factors influencing drug quality in the genus *Ocimum*

2.1. Genetic background

The essential oil's composition heritability is a genetically determined attribution. The exact mechanism of inheritance and genetic determination of basil is still not known. Alleles, which are situated in given locus of chromosome, are responsible for the formation of different properties, like essential oil compound and quantity, by coding of secondary metabolite proteins of *Ocimum*.

2.2. Ontogenic and morphogenic factors

NÉMETH (2005) mentioned that changes in essential oil composition are affected by enzyme activity or structural changes in tracts that accumulate the essential oil during ontogenesis in the Lamiaceae species. These changes are not influenced by genetic reasons but other parameters like abiotic factors, phase of development, harvesting time, and storage (HILTUNEN & HOLM, 1999).

LEMBERKOVICS and co-workers (1998) examined the change of essential oil composition of *O. basilicum* during the vegetation period. They concluded that basil oil was abundant in monoterpenes in budding and early flowering stages. Also established, that the quantity of sesquiterpenes and phenylpropane derivatives accumulated only in later stadiums.

The drug of basil is in its *herba,* so percentage of the organs may have influence on the drug quality, too.

MACHADO and co-workers (1999) examined the essential oils components of leaves and inflorescence oil of *O. tenuiflorum*. They discovered that the percentage of components varied in the different organs. Leaf oil contained eugenol (79.0–82.7%) and β-caryophyllene (7.9–9.8%) as main components, meanwhile inflorescence oil was abundant in eugenol (17.6–60.0%), β-caryophyllene (24.5–40.7%), and caryophyllene oxide (5.9–18.5%).

2.3. Environmental factors

Environmental factors can also affect the chemical composition of the essential oils as HILTUNEN and HOLM (1999) mentioned, although these changes particularly influence the accumulation of essential oil, as terpenes and phenylpropanes are generally under strict genetic control.

CHANG and co-workers (2005) reported that the temperature affect the composition of the essential oil. They observed that at warm temperature (25 °C) eugenol and *cis*-ocimene, under cooler conditions (15 °C) more camphor and *trans*-β-farnesene were accumulated in *O. basilicum* L. plant, meanwhile there was no effect of temperature on the relative content of 1,8-cineole and linalool.

Table 1. Chemical characterization of *Ocimum tenuiflorum*

Ocimum tenuiflorum	1,8-Cineole	β-Bisabolene	β-Caryophyllene	β-Elemene	Methyl chavicol	Methyl eugenol	Eugenol
Carović–Stanko and co-workers (2011)	5.14–24.90%	24.60–51.98%			3.35–21.62%		2.90–7.56%
Zheljazkov and co-workers (2008)					15–27%		8–43%
Pino and co-workers (1998)			23.1%	18.0%			34.3%
Raju and co-workers (1999)			31.7%	6.2%			53.4%
Raina and co-workers (2013)			4.13–44.60%	0.76–32.41%		0.87–82.98%	1.94–60.20%
Vani and co-workers (2009)			8.52–53.63%	1.59–6.35%		36.47–76.27%	
Wungsintaweekul and co-workers (2010)				9.38%		65.36%	
Jirovetz and co-workers (2003)			16.60%			56.18%	

Table 2. Chemical characterization of *Ocimum americanum*

Ocimum americanum	*trans*–β-Ocimene	Camphor	1,8–Cineole	Citral (neral +geranial)	Linalool	Terpinen-4-ol	(*E*) –Methyl cinnamate	(*Z*) –Methyl cinnamate	Eugenol
Yayi and co-workers (2001)					0.1–50.1%	7.5–52.8%			
Mondello and co-workers (2002)		38.6%							
Xaasan and co-workers (1981)		60.0%							
Tchoumbougnang and co-workers (2006)	29%		41.3%						
Hassane and co-workers (2011)			34.22–46.88%						
Sarin and co-workers (1992)				74.5%					
Saeio and co-workers (2011)				62.43%	5.51%				
Carović–Stanko and co-workers (2011)				40.44–59.97%	0–12.09%				
Carović–Stanko and co-workers (2010)				48.47%	12.15%				
Jirovetz and co-workers (2003)	5.95%						9.11%	72.05%	
Fun & Svendsen, (1990)			1.8–15%		4.8–8.9%		5.3–8.2%	38.1–65.2%	
Martins and co-workers (1999)								79.9%	
Vieira & Simon, (2000)							95.8%		
Gupta & Tava, (1997)					63.2%				
Ekundayo and co-workers (1989)									66.4%

Heavy shading significantly reduced the total essential oil content of fresh leaves as Chang and co-workers (2008) observed, however, young plants were more tolerant to shading than older plants. They also reported that higher irradiance significantly increased the relative contents of linalool and eugenol, whereas methyl eugenol increased by lower irradiance, however, the relative content of 1,8-cineol remained the same.

In the experiment of Khalid (2006), basil plants were treated with different levels of water stress, determined as a percentage of field water capacity by weight (50%, 75%, 100%, and 125%). These treatments were applied to investigate the change in the essential oil compounds in *O. basilicum* and *O. americanum.* According to the results, the water stress treatments increased the main components of essential oils. The effect of different treatments to the essential oil constituents may be due to their influence on the enzyme activity and metabolism.

Hassanpouraghdam and co-workers (2011) determined that salt content has a negative effect on the biosynthesis and accumulation of methyl chavicol, meanwhile the content of linalool was influenced positively by the integrated level of salt and zinc (200 mgl^{-1}). They also noticed that moderate salinity stress along with balanced level of foliar zinc application changed the primary metabolites pathway for the good of major essential oil compounds. Based on their findings, basil should be cultivated under semi-saline conditions.

2.4. Extraction of essential oil

In the literature, hydrodistillation and steam distillation are the ubiquitous methods to gain essential oil from basil. However, different extraction methods for the same plant can influence the quality of essential oil (Sefidkon et al., 2006).

In a recent study Carro and co-workers (2013) tested three extraction methods (steam distillation, ultrasound-assisted extraction, and microwave-assisted extraction) to maximize terpene extraction from *Ocimum basilicum* leaves. They observed that best result can be obtained with microwave-assisted extraction both in number of identified compounds and in their concentration.

2.5. Effect of drying and storage

During storage, the quantity of essential oils started to decrease in the dried basil samples in all the cases, worsening its quality parameters.

In the research of Baritaux and co-workers (1992), methyl chavicol, eugenol, linalool, and 1,8-cineole were identified as main components. The contents of methyl chavicol and eugenol decreased dramatically after drying and storage, in contrast to the linalool and 1,8-cineole contents, which increased at the same time. The total essential oil content showed a constant decreasing tendency during storage, because the two main compounds, methyl chavicol and eugenol, had suffered great losses.

Different drying methods also can influence the quality of essential oil components as found by Calín-Sánchez and co-workers (2012). In their experiment they tested the convective, vacuum microwave, and the convective pre-drying combined with vacuum microwave finish drying methods. Methyl eugenol, eugenol, eucalyptol, and linalool were the main components within the 40 compounds of sweet basil they identified. Their results showed that the total quantity of essential oil decreased in all samples independently of the utilized drying technique. The combined convective pre-drying – vacuum microwave finish drying technique produced the best results both in time required and aroma quality.

3. Conclusions

Basil is an important medicinal and culinary herb. Its essential oil contains several components like monoterpenes, sesquiterpenes, and phenylpropane derivates, which may be influenced by various factors like genetic background, ontogenesis, morphogenesis, environmental factors, method of essential oil extraction, drying, and storage.

The reason for variability on one hand could be that different plants with different origin from the same species were used in different experiments; on the other hand it could be caused by interspecific hybridization. Chemotype classification based on just one main essential oil component is problematic, because basil frequently contains two or more compounds in nearly equal quantity, also smaller components should be considered to be major compounds.

Although essential oils vary in different basil cultivars, main components are mainly oxygenated monoterpenes and phenylpropane derivates.

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