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Changes in the chemical composition of the essential oil of sweet basil (*Ocimum basilicum* L.) depending on the plant growth stage

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Genetic and environmental factors as well as plant ontogeny have a determining effect on the yield and quality of *Ocimum basilicum* L. volatile oil; ontogenetic variation is particularly important, since it largely determines the proper time for harvesting raw material as well as its chemical composition and activity. The aim of the present study was to determine relationships between the content and chemical composition of the essential oil of the sweet basil herb and the plant growth stage as well as to evaluate the usefulness of two basil cultivars for industry. The present experiment was conducted in a greenhouse during the period from February to May in the years 2008–2010. The basil herb was harvested at three growth stages: vegetative stage, flower bud stage, and full flowering, while the amount of essential oil (by hydrodistillation) and its composition (GC-MS and GC-FID) were evaluated in two cultivars: 'Kasia' and 'Wala'. The essential oil content in the herb of the basil cultivars under study was high (0.83% in the cultivar 'Kasia' and 0.75% in cv. 'Wala') and it increased with plant development. The studied essential oils were characterized by the presence of 63 compounds, among which linalool was the dominant one. The concentration of linalool was from 55.4% to 69.8%, depending on the cultivar and plant growth stage. The oil extracted at the flower bud stage was characterized by the highest proportion of linalool (in both cultivars) and of 1,8-cineole (only in the cultivar 'Kasia'). The concentration of methyl chavicol and methyl eugenol in the oil decreased together with the development of the basil plants studied, similarly to the concentration of limonene, α-humulene, *cis*-muurola-4(14),5-diene, and *trans*-calamene.

Key words: *Basilici herba*, basil cultivars, ontogenesis, linalool, 1,8-cineole, methyl chavicol

INTRODUCTION

Quantitative and qualitative variation of essential oil is caused by different factors, including genetic and ontogenetic ones. Within the species of *Ocimum basilicum* L., one can observe great differentiation not only in morphological characters of plants, but also in the content and chemical composition of essential oil. The divergence of morphological characters of sweet basil is extremely wide, similarly to the content of biologically active substances in its herb [1–3]. For this reason, numerous botanical forms and varieties of basil are distinguished; there are also many cultivars grown in different countries of the world for pharmaceutical, cosmetic, and food purposes. The basis for genotype classification of basil is, among others, the composition of its essential oil, the main biologically active substance of this plant. 18 basil genotypes are distinguished; they differ in the amount and quality of the dominant components of the essential oil, among others,

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estragole, methyl eugenol, linalool, methyl cinnamate or cineole, the proportion of which ranges from 10 to 86% [4].

The content of essential oil in basil and its chemical composition depend to a large extent on the location of a plantation, but they are also affected by genetic variation [5–8]. Among environmental factors, light and temperature have the greatest effect on the amount and composition of basil essential oil, which is associated with the tropical origin of this species [9–11]. Genetic variation, which is particularly strong in the genus *Ocimum* and which affects both morphological characters of plants and their chemical composition and properties, is equally important [8, 12–14]. In Europe the most important commercially species is *Ocimum basilicum* L. [1, 15, 16], whereas in the tropical regions of Africa the following species are of the greatest significance: *O. americanum* L., *O. basilicum* L., and *O. gratissimum* L. [5]. *O. kilimandscharicum* L., *O. lamiifolium* L., and *O. suave* L. [14], practically unknown in European cultivation, are also grown there. Raw material originating from the above-mentioned plants differs in the amount and composition of essential oil which is affected by both genetic and environmental factors. European basil varieties and forms belong to the linalool type, and the percentage of this component in the oil ranges from 46.9–48.9% [1, 15, 16] to 71.8% [17]. In turn, plants grown in the tropical climate (Africa) are characterized by the absence of linalool in the oil [14] or its medium content [5, 8]. A similar proportion of linalool in the volatile oil has been found in basil grown in India [12, 18]. The concentration of methyl chavicol, also called estragole, is also variable and shows a negative correlation with the concentration of linalool [2]. The proportion of the above-mentioned constituent in basil volatile oil is from 0 to 94.3% [6, 7, 19–21].

The relationship between age and essential oil content arises from the process of biosynthesis. Essential oil synthesis occurs only in very young cells; as the synthesis proceeds, the oil is released outside the cell. At the early period of leaf development, the release of oil is high and it remains at the same level during the further period of leaf growth, when leaf weight increases significantly. Therefore, the amount of oil in the leaf, expressed in percent, decreases with plant growth; this also results from losses caused by oil evaporation. The cultivar, cropping period, plant ontogeny, and plant part have a determining effect on the yield and quality of the volatile oil of *Ocimum basilicum* L. [3, 21–23]. Ontogenetic variation is particularly important, since it largely determines the proper time for harvesting raw material as well as its chemical composition and activity [21, 24–27]. Plants and their essential oils are a potential source of antimicrobial substances. The activity of individual volatile oils in relation to particular microorganisms is associated with their composition [8, 14, 23, 28], which is in turn determined by plant growth variation [21, 23, 24]. The essential oil obtained from *Basilici herba* is marked by various biological properties: antimicrobial [8, 14, 28], anticonvulsive [29, 30], and antioxidant [23, 31], and this oil can be used as a valuable therapeutic agent. It can also be perceived as a potential source of important, from the pharmacotherapeutic point of view, compounds such as linalool, eugenol, 1,8-cineole, or methyl chavicol. Linalool, as a compound with antimicrobial, antifungal, and antimalarial activity [32] as well as anti-inflammatory activity [33], seems to be a particularly valuable constituent of basil essential oil, especially when it is the dominant compound in it. European basil varieties are generally characterized by a high proportion of linalool in the oil [15–17], but one should take into account the effect of ontogenetic and environmental variation on the content of this component [10, 23, 34, 35]. The proportion of methyl chavicol, a compound with probable carcinogenic activity [36] whose concentration is negatively correlated with linalool content [2], in basil essential oil is equally important. Thus, the extraction of essential oil at an appropriate stage of plant development makes it possible to obtain the highest content of the desired components with special biological activity. The aim of the present study was (1) to determine relationships between the content and chemical composition of essential oil in the sweet basil herb and plant growth stage as well as (2) to evaluate two sweet basil cultivars in terms of the amount and quality of essential oil obtained from the herb harvested at different plant growth stages.

EXPERIMENTAL

Plant material and growing conditions

The experiment was conducted in the period from February to May 2008–2010, in a detached glasshouse of the Vegetable Crops and Medicinal Plants Department of the University of Life Sciences in Lublin. This facility is situated in the north-south direction. The temperature in the glasshouse ranged from 18–25 °C during the day and 12–15 °C at night. Basil was grown from seedlings produced from seeds of two Polish cultivars 'Kasia' and 'Wala' (obtained from the Institute of Natural Fibres and Medicinal Plants in Poznań). The morphological characteristics of the studied cultivars are presented in Table 1. Plants were grown in 4 dm^3 pots filled with sphagnum peat with a pH of 5.5–6.0. The experiment was set up as a completely randomized design in 8 replicates. One basil plant, being the experimental unit, was grown per pot. Basil seeds were sown on 28 February (2008), 12 March (2009), and 3 March (2010). Before sowing, seeds were treated (dressed) with the fungicide Dithane Neo Tec 75 WG. Seedlings emerged after 7–9 days. After about 18–20 days from sowing, the plants were thinned and transplanted into plug trays filled with peat substrate. The study was conducted under strictly controlled conditions. No disease symptoms or presence of pests were found on the plants during growth, therefore no plant protection agents were applied. The plants were placed in pots after about 25 days from sowing, at the fourth true leaf stage. The following amounts of nutrients, expressed in g per 1 dm^3 of substrate, were applied in the experiment: 0.6 N in the form of ammonium saltpeter;

0.8 K as potassium sulphate; 0.4 P as superphosphate 20% P; 0.3 Mg in the form of magnesium sulphate monohydrate, as well as the following micronutrients in mg per 1 dm^3 of substrate: 8.0 Fe [EDTA]; 5.1 Mn [$MnSO_4 \cdot H_20$]; 13.3 Cu [$CuSO_4 \cdot 5H_2O$]; 0.7 Zn [$ZNSO_4 \cdot 7H_2O$]; 1.6 B [H_3BO_3] and 3.7 Mo [$(NH_4)_6Mo_7)_{24} \cdot 4H_2O$]. During the experiment, the plants were watered with the same amount of water every 1–2 days. The plant harvest was conducted at the vegetative stage (VS) (about 21 days after the planting), the budding stage (BS) (about 28 days after the planting), and the beginning of flowering (BF) (about 40 days after the planting), by cutting off the above-ground part of the sprout above its lignified parts. Then, the herb was dried in a drying oven at a temperature of 35 °C and air-dry weight of the herb was determined.

Essential oil analysis

The volatile oil was extracted from the dried material by steam distillation of the herb for 3 hours. 20 g of raw plant material, together with approximately 400 ml of water, were submitted to water distillation. The oil preparations were made in three replicates. The oil obtained was collected in dark glass vessels, dried using dehydrated sodium sulphate and stored at below –10 °C until chromatographic determination.

Qualitative and quantitative analysis, GC-MS

A GC-MS instrument ITMS Varian 4000 GC-MC/MS (Varian, USA) was used, equipped with a CP-8410 auto-injector and a 30 m × 0.25 nm i. d. VF-5ms column (Varian, USA), film thickness 0.25 µm; carrier gas: helium at a rate of 0.5 ml/min; injector and detector temperature 220 °C and 200 °C, respectively; split ratio 1 : 100; injector volume 1 µl. A temperature gradient was applied (50 °C for 1 min, then incremented by 4 °C/min to 250 °C and held at this temperature for 10 min.); ionization energy 70 eV; mass range 40–1 000 Da; scan time 0.8 s. The linear retention indices from temperature-programming, using the definition of Van den Dool & Kratz [37], were determined for a series of *n*-alkanes (C_6–C_{40}).

GC-FID

A Varian 3800 Series (Varian, USA) instrument with a DB-5 column (J & W, USA) was used, operated under the same conditions as GC-MS; FID 260 °C, split ratio 1 : 50.

Qualitative analysis

The qualitative analysis was carried on the basis of MS spectra, which were compared with the spectra of the NIST library [28] and with data available in the literature [39]. The identity of the compounds was confirmed by their retention indices, taken from the literature [39] and from our own data. The quantitative composition of the volatile oil was determined using GC (FID) and by assuming that the total of the percentages of all oil components was 100%.

RESULTS AND DISCUSSION

The essential oil content in the herb of the studied basil cultivars ranged from 0.76% to 0.90% in the cultivar 'Kasia' and from 0.46% to 1.03% in cv. 'Wala' and it increased with plant development (Table 2). A different pattern of essential oil biosynthesis should also be noted in the basil cultivars under study. In the cultivar 'Kasia', the oil concentration increased relatively slowly (respectively: 0.76%, 0.83%, and 0.90%), but this process was much more dynamic in cv. 'Wala': at the vegetative stage, the herb was characterized by a lower amount of this substance by more than half (0.46%) of the amount at the initial period of flowering (1.03%). In turn, at the flower bud stage the herb contained nearly half less oil than at full bloom (respectively: 0.65% and 1.03%). The obtained results show that the process of essential oil biosynthesis in basil can be of different intensity, which is also affected by genetic factors. The stage of full development of inflorescences is considered to be optimal for harvesting the herb due to the highest essential oil content [21, 40]. On the other hand, harvesting the herb at the vegetative stage is the most popular method when basil is used as a fresh, aromatic spice. Evaluat-

Table 1. **Morphological characteristics of the studied sweet basil cultivars (mean for 2008–2010)**

Feature	'Kasia'	'Wala'
Plant height, cm	56.3	59.6
Plant diameter, cm	40.9	39.7
No. of branches, by plant	11.8	11.7
Length of leaf blade, cm	10.0	9.3
Width of leaf blade, cm	6.2	5.7
Fresh plant weight, g	93.1	84.1
Plant air-dry weight, cm	13.1	13.0
Plant habit	rounded	uplifted
Leaf colour	green	green

Table 2. **Essential oil content (% of ADW) of the studied sweet basil cultivars (mean for 2008–2010)**

'Kasia'			'Wala'		
VS	BS	BF	VS	BS	BF
0.76	0.83	0.90	0.46	0.65	1.03

VS – vegetative stage; BS – budding stage; BF – beginning of flowering.

ing the cultivars under study in this aspect, the cultivar 'Kasia' should be considered to have a greater value than 'Wala' due to nearly twice higher essential oil content in the herb of the former cultivar (respectively: 0.76% and 0.46%).

The investigated essential oils were characterized by the presence of 63 compounds, among which linalool was the dominant one (Table 3). The concentration of linalool was from 55.4% to 69.8%, depending on the cultivar and plant growth stage. The essential oil extracted from the herb of 'Kasia' plants contained more linalool (64.7%) than the oil obtained from the cultivar 'Wala' (60.5%), which is confirmed by the study of Dzida [17]. In the case of both oils

Table 3. **Chemical composition (% of ADW) of the essential oil from the herb of the studied sweet basil cultivars depending on the growth stage (mean for 2008–2010)**

No.	Compound	RI	'Kasia'			'Wala'		
			VS	BS	BF	VS	BS	BF
1	α-Pinene	941	0.2	0.3	0.1	0.3	0.3	0.3
2	Sabinene	979	0.2	0.2	0.1	0.3	0.3	0.3
3	β-Pinene	984	0.5	0.4	0.3	0.6	0.6	0.6
4	Myrcene	994	0.7	0.7	0.5	0.5	0.4	0.5
5	Limonene	1 033	0.2	0.1	0.1	0.4	0.3	0.2
6	1,8-Cineole	1 037	3.7	4.3	3.7	7.5	6.1	5.7
7	(*E*)-β-Ocimene	1 049	0.6	0.3	0.2	0.7	0.4	0.5
8	γ-Terpinene	1 061	tr	tr	tr	0.2	tr	tr
9	*cis*-Sabinene hydrate	1 076	0.1	tr	tr	0.3	tr	tr
10	fenchone	1 094	0.4	0.3	0.4	0.4	0.5	0.3
11	Linalool	1 105	60.9	69.7	63.6	55.4	68.9	57.2
12	Camphor	1 157	0.4	0.4	0.2	tr	0.3	0.2
13	δ-Terpineole	1 182	tr	tr	tr	tr	0.1	0.3
14	Terpinene-4-ol	1 192	tr	0.3	0.2	0.6	0.4	0.2
15	α-Terpineole	1 208	0.6	0.4	0.3	0.6	0.3	0.8
16	Methyl chavicol	1 213	tr	0.3	tr	1.1	0.8	0.6
17	Fenchyl acetate	1 226	0.2	tr	0.3	0.4	tr	tr
18	Geraniol	1 261	10.5	10.2	11.1	7.8	10.0	12.2
19	α-Cubebene	1 354	tr	tr	0.1	tr	tr	tr
20	Eugenol	1 362	0.4	0.2	0.2	0.1	0.3	0.1
21	α-Copaene	1 382	tr	tr	0.2	0.1	tr	tr
22	Geranyl acetate	1 388	tr	0.6	tr	0.8	1.1	1.9
23	β-Elemene	1 395	1.2	1.4	1.3	0.8	0.9	1.1
24	Methyl eugenol	1 418	3.4	0.4	0.6	1.4	1.1	0.1
25	(*E*)-Caryophyllene	1 430	0.5	0.4	0.5	0.3	tr	0.2
26	α-*trans*-Bergamotene	1 441	2.2	1.5	1.2	1.6	1.5	0.8
27	α-Guaiene	1 445	0.3	0.6	0.5	0.4	0.2	0.4
28	*cis*-Muurola-3,5-diene	1 458	0.3	0.3	0.3	0.1	0.4	0.3
29	α-Humulene	1 470	0.5	0.4	0.3	0.5	0.5	0.2
30	*cis*-Muurola-4(14),5-diene	1 476	1.1	0.9	0.6	1.0	1.0	0.6
31	β-Acoradiene	1 481	0.1	0.1	tr	tr	0.1	tr
32	β-Selinene	1 506	3.8	1.9	2.1	2.1	2.1	2.5
33	Bicyclogermacrene	1 511	0.1	tr	tr	0.4	0.3	0.3
34	α-Bulnesene	1 516	1.1	1.2	1.1	0.8	1.0	1.0
35	Germacrene A	1 523	0.9	1.0	0.9	0.5	0.4	0.6
36	γ-Cadinene	1 528	2.3	2.2	2.3	2.2	2.3	2.2
37	δ-Amorphene	1 531	tr	tr	tr	tr	tr	0.3
38	*trans*-Calamene	1 536	0.6	0.4	0.3	0.4	0.3	0.1
39	1,10-di-*epi*-Cubenol	1 630	0.9	0.8	1.2	0.7	0.7	0.8
40	*epi*-α-Cadinol	1 658	6.3	5.7	7.6	2.8	5.0	6.5
41	α-Cadinol	1 675	1.8	2.1	1.7	2.1	2.0	1.7
42	α-Bisabolol	1 711	0.1	tr	tr	tr	tr	tr

RI – non-isothermal Kováts retention indices (from temperature-programming, using the definition of Van den Dool and Kratz) for a series of *n*-alkanes (C_6–C_{40}); VS – vegetative stage; BS – budding stage; BF – beginning of flowering; tr – trace amount (<0.05%).

The following compounds were found in trace amounts: α-thujene, camphene, α-terpinene, terpinol, pinocarvone, *Z*-miroxide, nerol, neral, bornyl acetate, δ-elemene, β-cubebene, β-cedrene, β-(*Z*)-farnesene, germacrene A, 10-*epi*-cubebol, α-cadinene, longipinanol, spathulenol, caryophyllene oxide, globulol, viridiflorol.

in question, the concentration of linalool was the lowest at the vegetative stage, the highest at the budding stage, and it decreased with the development of flowers. The results of the study of Verma et al. [21] show high variations in the linalool content in the basil essential oil during plant ontogeny, but the highest concentration of this constituent was not always characteristic of the full flowering stage. Moreover, Chalchat et al. [22] showed different concentrations of linalool in the basil essential oil extracted from flowers, leaves, and stems. The proportion of the above-mentioned component in the basil oil also depends on seasonal variation [23]. Thus, in addition to ontogenetic variation, genetic and environmental variation seems to be important. A second component that was found in a significant amount in the studied basil essential oils was geraniol; its average percentage (10.3%) was nearly twice higher than that showed by Dzida [17] for the basil cultivars in question. The concentration of the above-mentioned component in the basil essential oil varies greatly, from trace amounts [20, 21] to 7.5%–61.3% [20, 41]. The proportion of geraniol in the studied essential oils changed during ontogeny; its highest content was found at full flowering (11.1% and 12.2%) as well as it increased with plant development (cv. 'Wala') (Table 3). Similar relationships were shown by Verma et al. [21] with respect to one of the cultivars under study during the spring and summer period.

The investigated basil essential oils were marked by a low proportion of methyl chavicol; its higher concentration was found in the cultivar 'Wala' compared to 'Kasia', which confirms the characteristics of these cultivars made by Dzida [17]. Methyl chavicol is one of more important components of the basil essential oil which is found in a very wide range, from 0.9% [12] to 93.3% [21]. Furthermore, the concentration of the above-mentioned constituent is different in particular parts of the herb: flowers, leaves, and stems [21, 22]. Methyl chavicol, as a compound that can have a carcinogenic effect [36], significantly affects the safety of the use of the basil oil in aromatherapy or in the perfume industry. The concentration of this compound in the essential oil extracted from the herb of the basil cultivar 'Wala' decreased with plant development and it was the lowest (0.6%) during the main stage (full flowering) as regards herb harvesting for pharmaceutical and industrial purposes. As far as the other cultivar under study is concerned, at the flowering stage the herb was characterized by a trace amount of methyl chavicol, likewise the herb at the vegetative stage (Table 3). Similar relationships were shown by Verma et al. [21] in the case of one of the studied cultivars during the autumn season. Another component found in larger amounts in the oil of the investigated basil cultivars was 1,8-cineole (eucalyptol). The concentration of 1,8-cineole was higher in the cultivar 'Wala' (6.4%) than in 'Kasia' (3.9%), which is confirmed by the study of Dzida [17]. The presence of 1,8-cineole in the basil essential oil is extremely important on account of its biological activity and the possibility to use it in pharmacotherapy [42, 43]. The concentration of eucalyptol in the basil oils under study was comparable to the content of the above-mentioned component in the essential oils of *Artemisia abiritium* L., *A. herbo-alba* Asso, *Mentha piperita* L., and *M. spicata* L. [36], mentioned as valuable medicinal plants. The percentage of 1,8-cineole in the volatile oil in the cultivar 'Kasia' was the highest at the flower bud stage (4.3%) and it was comparable in other growth stages. This was different in the case of cv. 'Wala', since the proportion of the said component decreased with plant development (Table 3). The study of Chalchat et al. [22] shows that the oil obtained from basil flowers and leaves does not contain eucalyptol, differently than the oil distilled from stems, which explains to some extent the relationships found in the present study.

The investigated basil volatile oils contained 4.8%–6.5% of *epi*-α-cadinol and 1.9% of α-cadinol, depending on the cultivar (Table 3). Dzida [17] gives similar values for the first of the above-mentioned compounds and much lower values for the other one. The concentration of these compounds changed during ontogeny. In the case of the cultivar 'Kasia', these changes were undirected, whereas in cv. 'Wala' the content of *epi*-α-cadinol increased with plant development, while the content of α-cadinol decreased (Table 3). Thus, it seems that the biosynthesis of the aforementioned sesquiterpene compounds may have a different pattern and that it is influenced by genetic and ontogenetic factors. The next group of components of the studied basil oils which were marked by a higher proportion included sesquiterpene hydrocarbons, among them germacrene D and γ-cadinene. The content of these compounds was in the range from 2.1% to 3.8% and it changed differently during the growth of the plants under study. These changes were generally undirected, except for the concentration of germacrene D in the oil extracted from the herb of 'Wala' which increased with inflorescence development. The proportion of germacrene D in the basil essential oil is clearly dependent on ontogenetic factors [21–23], but genetic factors are also important [21]. The basil essential oils studied contained on average 0.9%–1.5% of methyl eugenol and 1.3%–1.7% of α-*trans*-bergamoten, while the concentration of the above-mentioned compounds was higher in the cultivar 'Kasia' than in 'Wala' and it decreased with plant development – similarly to the concentration of eugenol the content of which in the investigated oils was low. Similar correlations between ontogeny and changes in the content of methyl eugenol and eugenol are shown by Verma et al. [21]. The decrease in the concentration of methyl eugenol in the basil oil with plant growth should be considered to be important, due to its possible carcinogenic activity [44].

The content of the other constituents of the essential oil of the basil cultivars under study was lower than it was in the case of the above described components and it was differently variable during ontogeny. With plant development, the content of limonene, α-humulene, *cis*-muurola-4-(14),5-diene, and *trans*-calamene decreased in the analysed

oils. Similar correlations relating to the concentration of limonene and α-humulene were shown in an earlier study [24]. Changes in the concentration of the other constituents were different in the cultivars studied and at the plant growth stages in question.

CONCLUSIONS

The present study showed high usefulness of the basil cultivars under investigation for both the pharmaceutical and perfume industries as well as for the food industry due to their high content of linalool, a low proportion of methyl chavicol as well as the presence of geraniol, 1,8-cineole, and germacrene D. The cultivar 'Kasia' was characterized by a higher content of essential oil in the herb, a higher concentration of linalool, and a low proportion of methyl chavicol compared to the cultivar 'Wala'. It should be stressed that the studied cultivars had a high content of essential oil whose composition underwent certain changes during ontogeny. The oil extracted at the flower bud stage was characterized by the highest proportion of linalool (in both cultivars) and of 1,8-cineole (only in cv. 'Kasia'). With plant development, the concentration of methyl chavicol and methyl eugenol decreased; these are compounds that greatly limit the use of basil oil due to their possible mutagenic activity.

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S a n t r a u k a

Buvo tiriama saldžiojo baziliko eterinių aliejų sudėtis skirtingais augalo vegetacijos laikotarpiais. Tirti šiltnamyje auginti augalai 2008–2010 m. vasario–gegužės mėn. 'Kasia' nustatytas didesnis kiekis eterinių aliejų, palyginti su 'Wala' veisle. Pateikta išsami eterinių aliejų sudėtis.