

## GC-MS ANALYSIS OF THE ESSENTIAL OIL OBTAINED FROM *OCIMUM BASILICUM* L. “HOLLAND” CULTIVAR

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### Abstract

This paper presents the quantitative and qualitative analysis of the essential oils obtained from a “Holland” cultivar of *Ocimum basilicum* L (basil). The vegetal material was harvested from the experimental fields of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca (Romania) at different times of the year: June (sample 1), August (sample 2) and September (sample 3), 2008. The essential oils obtained by steam distillation were analyzed by gas-chromatography coupled with mass spectrometry (GC-MS). The content in essential oils ranged from 0.4% to 0.8%; the maximum amounts were observed in June while the minimum in September. The most important components identified in essential oils were: linalool,  $\alpha$ -cadinol, trans- $\alpha$ -bergamotene,  $\gamma$ -cadinene,  $\beta$ -elemene, methylchavicol and delta-guaiene. We can consider that the „Holland” cultivar of *Ocimum basilicum* may be considered as „linalool-rich” chemotype [10], because for all essential oil samples the major constituent was linalool. The amount of essential oil of basil, and the chemical composition depend on the harvesting time, June being the optimal period for the volatile oil content (0.8%) and August for linalool.

### Rezumat

În această lucrare se prezintă analiza calitativă și cantitativă a uleiurilor volatile obținute de la *Ocimum basilicum* L. (*Lamiaceae*), varietatea “Holland” (busuioc). Materialul vegetal a fost recoltat de pe câmpurile experimentale ale Universității de Științe Agricole și Medicină Veterinară Cluj-Napoca (România) în lunile iunie (proba 1), august (proba 2) și septembrie (proba 3), anul 2008. Uleiurile volatile s-au obținut prin distilare cu vapori de apă și analizate prin gaz-cromatografie cuplată cu spectrometrie de masă (GC-MS). Conținutul în ulei volatil a fost 0,4 -0,8%, cantitatea maximă fiind obținută de la materialul recoltat în luna iunie, iar minimă s-a înregistrat în septembrie. Componentele cele mai importante identificate în uleiurile volatile au fost: linalool,  $\alpha$ -cadinol, trans- $\alpha$ -bergamoten,  $\gamma$ -cadinen,  $\beta$ -elemen, metilchavicol, delta-guaien. Pentru că în toate probele, constituentul majoritar a fost linaloolul, se poate considera că varietatea „Holland” se încadrează în chemotipul bogat în linalool [10]. Concentrația de ulei volatil din busuioc, precum și compoziția sa, depind de perioada de recoltare, luna iunie fiind perioada optimă pentru conținutul în ulei volatil (0,8%), iar luna august pentru linalool.

**Keywords:** cultivar, *Ocimum basilicum*, GC-MS, linalool.

## Introduction

Basil (*Ocimum basilicum* L., *Lamiaceae*) is as well known aromatic and medicinal plant, spread in Asia and cultivated in all the Mediterranean and tropical countries [4,5,9]. The chemical composition of essential oils depends on the chemotype and the development stages [2,9,11,13]. There are known several *Ocimum basilicum* chemotypes, depending on major compounds of the essential oil: linalool for the Southern Europe and Egypt; methylcinnamate and linalool for tropical areas; methylchavicol or methyleugenol for Madagascar and Comoro Island [4,9,14]. On the basis of more than 200 analyses of essential oils isolated from *O. basilicum* L., Lawrence classified four major essential oil chemotypes of basil: (1) methylchavicol-rich, (2) linalool-rich, (3) methyleugenol-rich, (4) methylcinnamate-rich, and also numerous subtypes [10]. Within the *Ocimum* species, the essential oil composition differs significantly, depending on the geographical origins, different regions of the plant and developmental stages [4,10,11].

The aim of our study was to analyze the chemical composition of essential oils obtained from the "Holland" variety of *Ocimum basilicum* harvested at different times of the year: June, August and September, 2008. This variety was cultivated in the experimental fields of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, using seedling plants obtained in plastic cups. The transplant plantation was performed at 50 cm row distance and 25 cm between plants [11].

## Materials and Methods

Three samples of basil essential oil, obtained from a new "Holland" cultivar of *Ocimum basilicum*, were analyzed by gas-chromatography coupled with mass spectrometry (GC-MS). The aerial parts of the plants were harvested from experimental fields of the University of Agricultural Sciences and Veterinary Medicine from Cluj at different times of the year: in June (sample 1), in August (sample 2) and September (sample 3), 2008.

The essential oils (*Basilici aetheroleum*) were obtained by steam distillation of the dried material in accordance with the Romanian Pharmacopoeia [16]. The distilled oils were dried over anhydrous sodium sulfate and stored in tightly closed dark vials at 4°C until the analyses were carried out [12,16].

*Essential oil analysis procedure:* The three samples were diluted with cyclohexane in a 1:1 proportion and a volume of 0.1 µL was injected. An Agilent 6890 Series gas-chromatograph coupled with a Hewlett Packard 5973 mass spectrometer, and a capillary Permabond column SE-52, (60m x

0.25mm, 0.25 $\mu$ m) were used. The injector and detector temperatures were kept at 250°C and 280°C, respectively. Helium was used as a carrier gas, with a flow rate of 0.6 mL/min; oven temperature programmed was 60-240°C at a rate of 3°C/min. Mass spectrometry (MS) analysis was performed in the same conditions, with an ionization voltage of 70eV and mass range was from 35-700 u.i.m. [6].

Identification of individual compounds was performed by comparison of their mass spectra with those of the internal reference mass spectra libraries (Wiley 275 and NIST 98). Retention indices were calculated using a modified Van den Dool and Kratz formula [15], by interpolation of retention indices of C<sub>9</sub>-C<sub>26</sub> n-alkanes, under the same conditions as described for the GC analysis. Identification was confirmed by comparison of the retention indices with those described in the Adams library [1].

### Results and Discussion

The essential oils' level (mL essential oil/100 g natural product $\pm$ SD) for the three samples was determined by steam distillation: 0.80 $\pm$ 0.07 (sample 1 harvested in June), 0.60 $\pm$ 0.04 (sample 2, August) and 0.40 $\pm$ 0.01 (sample 3 from September). The results obtained represent the mean of three determinations $\pm$ SD. Considering the quantity of essential oils, June was the optimal period for harvesting the natural product. In literature, the reported levels of essential oil in *Ocimum basilicum* are between 0.2% and 1% [4,7,8].

The compounds identified in the essential oils of *O. basilicum* L. and their levels are presented in table I (percentage value represents the mean of three determinations $\pm$ SD).

**Table I**  
Chemical composition of *Basilici aetheroleum*

| Compounds                   | R <sub>T</sub> | Essential oil (%)<br>from sample 1 | Essential oil (%)<br>from sample 2 | Essential oil (%)<br>from sample 3 |
|-----------------------------|----------------|------------------------------------|------------------------------------|------------------------------------|
| limonene (MH)               | 15.934         | 0.062 $\pm$ 0.01                   | 0.113 $\pm$ 0.01                   | 0.102 $\pm$ 0.01                   |
| trans $\beta$ -ocimene (MH) | 16.650         | 0.039 $\pm$ 0.01                   | 0.148 $\pm$ 0.01                   | 0.123 $\pm$ 0.01                   |
| linalool (OM)               | 19.032         | 21.053 $\pm$ 1.17                  | 38.540 $\pm$ 2.67                  | 22.478 $\pm$ 1.73                  |
| borneol (OM)                | 22.277         | 0.387 $\pm$ 0.02                   | 0.162 $\pm$ 0.01                   | 0.130 $\pm$ 0.01                   |
| $\alpha$ -terpineol (OM)    | 23.344         | 0.601 $\pm$ 0.05                   | 0.398 $\pm$ 0.03                   | 0.581 $\pm$ 0.05                   |
| methylchavicol (AT)         | 23.724         | 0.132 $\pm$ 0.01                   | 5.890 $\pm$ 0.56                   | 0.818 $\pm$ 0.07                   |
| geraniol (OM)               | 26.034         | 0.676 $\pm$ 0.04                   | 0.941 $\pm$ 0.08                   | 1.348 $\pm$ 0.11                   |
| geranial (OM)               | 26.923         | -                                  | 0.044 $\pm$ 0.01                   | 0.133 $\pm$ 0.01                   |
| bornyl acetate (OM)         | 27.686         | 1.135 $\pm$ 0.12                   | 0.391 $\pm$ 0.02                   | 1.249 $\pm$ 0.12                   |
| $\alpha$ -cubeben (SH)      | 30.507         | 0.081 $\pm$ 0.01                   | 0.143 $\pm$ 0.01                   | 0.186 $\pm$ 0.01                   |
| eugenol (AT)                | 30.857         | 0.628 $\pm$ 0.05                   | 0.444 $\pm$ 0.05                   | 0.212 $\pm$ 0.01                   |
| $\alpha$ -copaen (SH)       | 31.749         | 0.408 $\pm$ 0.04                   | 0.666 $\pm$ 0.06                   | 0.789 $\pm$ 0.08                   |

| Compounds                           | R <sub>T</sub> | Essential oil (%)<br>from sample 1 | Essential oil (%)<br>from sample 2 | Essential oil (%)<br>from sample 3 |
|-------------------------------------|----------------|------------------------------------|------------------------------------|------------------------------------|
| β-bourbonen (SH)                    | 32.202         | 0.197±0.01                         | 0.145±0.01                         | 0.287±0.02                         |
| β-elemene (SH)                      | 32.407         | 7.083±0.72                         | 6.638±0.59                         | 7.065±0.71                         |
| trans-caryophyllene (SH)            | 33.737         | 1.315±0.15                         | 1.613±0.11                         | 1.006±0.12                         |
| trans-α-bergamotene (SH)            | 34.190         | 7.676±0.82                         | 4.975±0.41                         | 5.017±0.48                         |
| α-guaiene (SH)                      | 34.395         | 3.500±0.29                         | 3.273±0.25                         | 3.689±0.35                         |
| β-farnesene (SH)                    | 34.833         | 0.354±0.02                         | 0.209±0.02                         | -                                  |
| α-humulene (SH)                     | 35.169         | 0.952±0.07                         | 0.845±0.08                         | 1.045±0.12                         |
| epi-bicyclo-sesquiphellandrene (SH) | 35.535         | 1.109±0.09                         | 0.860±0.07                         | 1.021±0.11                         |
| germacrene D (SH)                   | 36.295         | 4.737±0.46                         | 5.255±0.52                         | 5.013±0.47                         |
| β-selinene (SH)                     | 36.543         | 0.550±0.04                         | -                                  | 0.482±0.03                         |
| δ-guaiene (SH)                      | 37.245         | 6.503±0.62                         | 5.597±0.49                         | 6.207±0.61                         |
| γ-cadinene (SH)                     | 37.596         | 7.198±0.80                         | 4.232±0.34                         | 7.026±0.69                         |
| β-sesquiphellandrene (SH)           | 37.798         | -                                  | 0.149±0.01                         | 0.142±0.01                         |
| δ-cadinene (SH)                     | 37.859         | 0.505±0.04                         | 0.409±0.03                         | 0.559±0.04                         |
| cis-calamenene (SH)                 | 37.947         | 0.823±0.05                         | 0.283±0.01                         | 0.748±0.06                         |
| nerolidol-E (OS)                    | 39.218         | 0.310±0.03                         | 0.134±0.01                         | 0.411±0.04                         |
| spathulenol (OS)                    | 40.197         | 0.207±0.01                         | 0.062±0.01                         | 0.423±0.03                         |
| α-cadinol (OS)                      | 42.595         | 16.502±1.16                        | 8.247±0.73                         | 14.431±1.43                        |
| α-bisabolol (OS)                    | 43.983         | 0.071±0.01                         | 0.166±0.01                         | 0.032±0.01                         |
| hexahydrofarnesylacetone (OS)       | 49.348         | 0.799±0.07                         | 0.261±0.02                         | 0.755±0.05                         |

Note: MH-monoterpene hydrocarbons, OM-oxygenated monoterpenes, SH-sesquiterpene hydrocarbons, OS-oxygenated sesquiterpenes, AT-aromatic terpenes; R<sub>T</sub> -retention time

In the essential oils obtained from samples 1 and 3 we identified 41 compounds, representing 86.432% and respectively 84.104%, whilst in sample 2, 45 compounds were identified (representing 92.734%).

Linalool (21.053-22.478%), α-cadinol (16.502-14.431%), trans-α-bergamotene (7.676-5.017%) were found as the major compounds in the samples 1 and 3 (Table I). The analyzed essential oils mainly consisted of sesquiterpenes hydrocarbons (50.15-47.96%) followed by oxygenated monoterpenes (27.80-30.96%), oxygenated sesquiterpenes (20.69-19.08%), aromatic terpenes (0.98-1.34%) and monoterpenes hydrocarbons (0.35-0.64%). Between the chemical composition of essential oils obtained from samples 1 and 3 there are small differences (not statistically significant).

Linalool (38.540%), α-cadinol (8.247%), β-elemene (6.638%), methylchavicol (5.890%) were found as the most important compounds in sample 2 (Table I). The identified constituents were oxygenated monoterpenes (44.86%), sesquiterpenes hydrocarbons (38.13%), oxygenated sesquiterpenes (9.56%) aromatic terpenes (6.83%) and monoterpenes hydrocarbons (0.61%). Methylchavicol was found in quite large quantities (5.890%) in sample 2, by comparison with other samples

(0.132% in sample 1 and 0.818% in sample 3). The Committee on Herbal Medicinal Products (HMPC) stated in a rapport on the use of natural products containing estragole, that exposure to estragole (short time use of medicinal products in adults at recommended posology) does not pose a significant cancer risk [17].

Because of the results obtained by GC-MS on the essential oil samples of basil, the „Holland” cultivar of *Ocimum basilicum* may be considered as „linalool-rich” chemotype [10]. In previous researches on basil essential oil of Romanian origin, linalool was the main compound [3,7] whereas in other samples methylchavicol (33.76%) and linalool (24.76%) were the main compounds [8].

### Conclusions

The quantitative analysis that was performed, showed a higher level of essential oil in the dried material obtained from plants harvested in June. Linalool was the major compound identified in all samples. August can be considered the best period to harvest the medicinal product in order to obtain linalool in higher quantities.

Considering the quantity (0.8%) and quality (38.54% linalool) of essential oils, the “Holland” cultivar of *Ocimum basilicum* can be cultivated in the climate and soil conditions of Romania and can be turned to account as a flavouring and therapeutical agent.

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