Study of the changes in the chemical composition of Bulgarian dill essential oils

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Abstract. The chemical composition and therefore the quality of essential oils can vary depending on several factors like the time of harvest, the location of the crop, the part of the plant, the geographical and climatic conditions as well as the production method. The aim of this work is to study the change in the composition of the Bulgarian dill essential oils of known origin during the different harvest years and its storage. Plant parts of dill (Anethum graveolens L.) from the region of village Gavrailovo, Bulgaria were investigated. Significant qualitative and quantitative differences in the chemical composition of the studied oils and the data in the literature were detected. It has been established that the essential oils from the herb and the fruits of the dill in the region of village Gavrailovo contains significant amounts of methyl chavicol (32,90 – 62.96 %) which is the reason to be considered as a new methyl chavicol chemotype.

1. Introduction

The composition of essential oils is significantly determined by the geographical and climatic conditions in which the aromatic plants were cultivated. Two types of essential oils are obtained from the dill (Anethum graveolens L.) – from the overground parts (weed oil) and from the fruits (seed oil). The two oils differ in aromatic components, color, taste, odor and application [1].

The weed oil is produced in larger quantities than the seed oil. The main manufacturers are the United States, France, Hungary and Eastern European countries in quantities of about 100 tons/year. The weed oil is an easy flowing, light yellow liquid with a specific odor. About 53 components (mono- and sesquiterpenes, and phenols) and 25 acids have been identified in the composition of the weed oil. Determining smell and taste are: phellandrene, limonene, oxy-p-menth-1-ene and carvone. The main components are: α-phellandrene (17.0 - 56.4 and even up to 66.5 %), limonene (5.7 - 44.9 %), carvone (4.3 - 54.5 %), 3,9-oxy-p-menth-1-ene (2.8 - 21.6 and up to 37.5 %), dihydrocarvone (up to 16.0 %). It also contains β-phellandrene (0.3 - 8.5 %), p-cymene (0.1 - 4.2 and even 14.4 %), α-pinene (1.0 - 3.2 %), dillapiole and myristicin (about 0.3% each), β-myrcene, camphene, terpinolene, carvacrol, 1,8-cineole, germacrene D and others [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14].

Georgiev and Dimitrov (1982) established for the first time the presence of dihydrocarveol in the Bulgarian dill oil [15].

For Bulgarian dill weed oil typical components (BDS 17380:1996) are: α-phellandrene (17.0 – 32.0 %), limonene (17.0 – 32.0 %), dihydrocarvone (4.0 – 9.0%) and carvone (25.0 – 45.0%) [1]. In the oil in the region of Yambol (42° 35’ N and 26° 53’ E) Dimov at al. [16] found the absence of dihydrocarvone, low content of carvone (8,4%) and high of carvacrol (20,9%).

The seed essential oil is easy flowing, dark yellow liquid with a characteristic odor like a carvone, and spicy taste. Four chemotype oils has been identified [1]:
I chemotype – limonene (36.9 – 46.7 %), carvone (17.8 – 45.6 %), myristicin (0.2 – 20.3 %) and dillapiol (8.0 – 2.3 %);  
II chemotype – limonene (31.0 – 40.9 %), carvone (25.1 – 47.4 %) and dillapiol (6.3 – 31.8 %);  
III chemotype – limonene (39.5 – 50.7 %) and carvone (43.7 – 57.7 %);  
IV chemotype (from the Balkan countries) – limonene (about 43 %), carvone (about 41 %) and myristicin (about 11 %).  

About 40 components have been identified in the seed oil, the amount of which varies considerably depending on the degree of maturity of the fruits, the sort and the soil-climatic conditions of the plant growing place. Determining the odor and taste of the oil are limonene and carvone. The main components of seed oil are: limonene (9.0 – 3.0 and sometimes up to 68.0 %), carvone (6.0 – 36.0 %), dihydrocarvone (0.9 – 7.8 and up to 18 %), α-phellandrene (0.2 – 38.0 %), oxy-p-menth-1-ene (0.3 – 17.0 %), carveol and dihydrocarveol (0.1 to 4.0 %), apiole (0.1 – 4.0%), dillapiole (up to 3.0 %), myristicin (up to 3.2 and sometimes up to 11.7 %), isoeugenol, α- and β-pinene, α-terpinene, terpinene-1-ol-4, etc. [1, 2, 3, 14, 17, 18, 19, 20, 21, 22, 23].  

In our advanced research was found that the essential oils from the herb and the fruits of the Bulgarian dill from the region of Gavrailovo (42° 38’ N and 26° 11’ E) contains significant amounts of methyl chavicol, aromatic compound found in tarragon (Artemisia dracunculus L.), basil (Ocimum basilicum L.) and anise (Pimpinella anisum L.) oils. There is no other literature data to support this, which is a reason to conduct more in-depth research. The aim of this work is to study the change in the composition of the Bulgarian dill essential oils of known origin during the different harvest years and its storage.

2. Materials and Methods

2.1. Plant material
The plant parts of a dill (Anethum graveolens L.) were collected in the vicinity of the village Gavrailovo (42° 38’ N and 26° 11’ E), Sliven, Bulgaria of 2018 and 2020 growing seasons. The dried overground parts of the plant and the fruits have been studied. The plant parts were air-dried immediately after harvesting in a shady site for 10 days, packed in paper bags and kept in a dark, dry and cool place. The raw material was stored under standard conditions. The moisture content of the fresh raw materials was determined by drying to constant weight, at 105 °C [24].

2.2. Essential oil extraction
The essential oil is extracted by hydrodistillation in a laboratory device of the British Pharmacopoeia, modified by Balinova and Dyakov, % w/v [25]. The distillation begins with the separation of the first drop of distillate and pouring it into a container. Distillation is complete when two consecutive measurements in 30 min do not mark an increase in the amount of essential oil. The oil obtained was dried over anhydrous sodium sulfate and stored in tightly closed dark vials at 4°C until analysis.

2.3. Gas Chromatography (GC) and Gas Chromatography/Mass Spectrometry (GC/MS) analyses.
The chemical composition of the essential oils is determined by gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS) by direct headspace analysis according to ISO standards (ISO 11024-1: 1998, ISO 11024-2: 1998).

**GC analysis:** Agilent 7890 A device with flame ionization detector; HP-INNOWax Polyethylene Glycol Column (60 m x 0.25 mm; 0.25 μm film); temperature conditions: 70 °C for 10 min, 70 - 240 °C at 5 °C/min, 240 °C for 5 min; 240-250 °C at 10 °C/min, 250 °C for 15 min; helium carrier gas, 1 cm³/min constant velocity; injector: split, 250 °C, split ratio 50:1.

**MS/GC analysis:** Agilent 5975 C device, helium carrier gas, column and temperature conditions as in the GC assay; detectors: FID, 280 °C, MSD, 280 °C transfer line. The flavor components are identified by comparison with the witness retention index and mass spectra (MS), stacked at retention time, the amount is given in percent.
The identification of chemical compounds was made by comparison to their relative retention time and library data. The components identified were arranged according to the retention time and their quantity was given in percentage.

2.4. Statistical analysis
All experiments were performed in triplicate, with values in the tables and graphs averaged, and represented with their mean and standard deviation. The obtained measurements and calculations were processed in MS Excel ver. 2016 (Microsoft Corporation Inc.) at a level of significance \( \alpha=0.05 \).

3. Results and discussion:
The essential oils from dill herb and fruits from 2018 and 2020 harvest years, as well as from stored raw material were obtained. The essential oil yields have been shown in Table 1. It was found that 64.25% of the content of weed essential oil is preserved during the storage of the raw material for a period of two years. For fruits, this amount is 77.88%. This is explained by the way the essential oil is distributed in the different parts of the plant. As seen, weed oil yield for 2020 is twice as high as for 2018. The difference in essential oil yields may be due to environmental conditions under which the plant has grown. The seed oil contents for the two harvest years (2018 and 2020) were approximately the same.

The chemical composition of the obtained essential oils was determined (Table 2) and compared with the data from the literature.

### Table 1. Content of essential oil in herb and fruits of Bulgarian dill.

| Essential oil, % (v/w) | 2018 harvest year | after 2 years of storage | 2020 harvest year |
|------------------------|-------------------|--------------------------|-------------------|
| Dill weed oil          | 2.21 ± 0.05       | 1.42 ± 0.03              | 4.40 ± 0.08       |
| Dill seed oil          | 9.99 ± 0.11       | 7.78 ± 0.02              | 8.47 ± 0.03       |

The chemical composition of the obtained essential oils was determined (Table 2) and compared with the data from the literature.

### Table 2. Chemical composition of dill essential oils during different harvest years and after storage.

| № | Components                  | RI* | 2018 harvest year | 2020 harvest year |
|---|-----------------------------|-----|-------------------|-------------------|
| 1 | α-Thujene                   | 924 | 0.37              | -                 | 0.26              | 0.14              |
| 2 | α-Pinene                    | 939 | 1.14              | 1.39              | 1.30              | 2.05              |
| 3 | Camphene                    | 946 | 0.20              | -                 | 0.23              | 0.21              |
| 4 | 2-(E)-Hepten-1-ol          | 958 | -                 | 1.92              | -                 | -                 |
| 5 | Sabinene                    | 969 | 0.26              | -                 | 0.26              | 0.80              |
| 6 | β-Pinene                    | 979 | 0.31              | 0.57              | 0.19              | 0.20              |
| 7 | Myrcene                     | 988 | 1.67              | 0.91              | 1.57              | 0.98              |
| 8 | α-Phellandrene              | 998 | 10.71             | 0.78              | 19.38             | 10.33             |
| 9 | α-Terpinene                 | 1015| -                 | -                 | 1.83              | 0.10              |
| 10| p-Cymene                    | 1024| 12.65             | 1.88              | 0.36              | 0.53              |
| 11| β-Phellandrene              | 1028| 3.23              | -                 | -                 | -                 |
| 12| Limonene                    | 1030| 4.86              | 3.22              | 3.44              | 2.66              |
| 13| 3-E-Octen-2-one             | 1032| -                 | 4.03              | -                 | -                 |
As seen 17 components representing 97,40% of the total content were identified in the weed oil (2018). 10 of them were in concentrations over 1% and the rest 7 – in concentrations under 1%. As seen the main constituents (over 3%) of the oil are as follows: methyl chavicol (32.90%), p-cymene (12.65%), α-phellandrene (10.71%) carvone (6.42%), limonene (4.86%) and carvacrol acetate (3.25%).

In the seed essential oil (2018) 15 components have been identified (98.16% of all components). 10 of them were in concentrations over 1% and the rest 5 – in concentrations under 1%. The main components (over 3%) are as follows: methyl chavicol (62.96%), fenchone (12.65%), 3-E-Octen-2-one (4.03%) and limonene (3.22%).

After two years of storage of the raw material, in the weed oil 16 components have been identified (98.05% of all components). 9 of them were in concentrations over 1% and the rest 7 – in concentrations under 1%. The main components (over 3%) are as follows: methyl chavicol (56.72%), fenchone (19.05%), limonene (4.88%) p-cymene (4.46%) and α-pinene (3.60%). In the seed oil from the stored raw material 15 components have been identified (97.67% of all components). The main components are the same: methyl chavicol (54.88%), fenchone (18.10%), limonene (5.64%) p-cymene (5.24%) and α-pinene (4.42%). During storage, the odor characteristics of the two oils converge.

17 components representing 98.64% of the total content were identified in the weed oil (2020). 9 of them were in concentrations over 1% and the rest 8 – in concentrations under 1%. As seen the main components (over 3%) of the oil are as follows: methyl chavicol (49.37%), α-phellandrene (19.38%), fenchone (12.61%), limonene (3.44%) and exo-fenchyl acetate (3.25%).

In the seed essential oil (2020) 17 components have been identified (97.33% of all components). 7 of them were in concentrations over 1% and the rest 10 – in concentrations under 1%. The main components (over 3%) are: methyl chavicol (58.49%), fenchone (12.22%), α-phellandrene (10.33%) and γ-terpinene (6.37%).

The data has shown that the oils cannot be attributed to the described chemotypes [1]. The difference in chemical composition of our investigations and the reported data may be due to environmental conditions under which the plant has grown, as well as from the origin of the seed for sowing.

The classification of the identified compounds, based on functional groups, is presented in figure 1 for weed oils and in figure 2 for seed oils respectively.

|   | Eucalyptol | β-cis-Ocimene | 2-E-Octen-1-al | γ-Terpinene | 2-E-Octen-1-ol | Fenchone | Methyl chavicol | cis-Cardanol | Fenchyl acetate | Anethol | Carvacrol acetate |
|---|------------|---------------|----------------|-------------|----------------|-----------|----------------|-------------|----------------|---------|------------------|
| 14| -          | -             | -              | -           | 1025           | 0,74      | 0,70           | 1,79        | 1,59           | -       | -                |
| 15| -          | -             | -              | -           | 1031           | nd        | nd             | 0,25        | 0,05           | -       | -                |
| 16| -          | -             | 1048           | 0,53        | 1053           | 1,73      | 1,64           | 0,80        | 6,37           | -       | -                |
| 17| -          | -             | 1054           | 1,54        | -              | -         | -              | -           | -              | -       | -                |
| 18| -          | -             | 1060           | -           | 1082           | 19,05     | 18,10          | 12,61       | -              | -       | -                |
| 19| -          | -             | 1085           | 2,61        | 12,65          | 1082      | 19,05          | 18,10       | 12,61          | -       | -                |
| 20| -          | -             | 1193           | 1,54        | 62,96          | 1195      | 56,72          | 54,88       | 49,37          | 58,49   | -                |
| 21| -          | -             | 1226           | -           | 0,16           | -         | -              | -           | -              | -       | -                |
| 22| -          | -             | 1239           | 0,39        | 0,24           | -         | -              | -           | -              | -       | -                |
| 23| -          | -             | 1229           | 0,16        | 1250           | 1,83      | 1,74           | 0,36        | 0,28           | -       | -                |
| 24| -          | -             | 1282           | 0,10        | -              | 1250      | 1,83           | 1,74        | 0,36           | 0,28    | -                |
| 25| -          | -             | 1370           | 3,25        | -              | -         | -              | -           | -              | -       | -                |

RI* = Relative Index

As seen 17 components representing 97,40% of the total content were identified in the weed oil (2018). 10 of them were in concentrations over 1% and the rest 7 – in concentrations under 1%. As seen the main constituents (over 3%) of the oil are as follows: methyl chavicol (32,90%), p-cymene (12,65%), α-phellandrene (10,71%) carvone (6,42%), limonene (4,86%) and carvacrol acetate (3,25%).

The classification of the identified compounds, based on functional groups, is presented in figure 1 for weed oils and in figure 2 for seed oils respectively.
Figure 1. Groups of components in dill weed oils, %:
1 – monoterpene hydrocarbons; 2 – oxygenated monoterpenes; 3 – sesquiterpene hydrocarbons; 4 – phenyl propanoids; 5 – aliphatic hydrocarbons; 6 – oxygenated aliphatic.

Phenyl propanoids (50.60 %) are the dominant group in the weed oil from 2018, followed by monoterpene hydrocarbons (23.90%), oxygenated monoterpenes (15.82 %), sesquiterpene hydrocarbons (6.03 %), aliphatic hydrocarbons (2.90 %) and oxygenated aliphatic (0.75 %). In the weed oil after storage dominant group again are phenyl propanoids (58.55 %), followed by oxygenated monoterpenes (20.40 %) and monoterpene hydrocarbons (19.15 %). Phenyl propanoids (49.73 %) are the dominant group and in the weed oil from 2020, followed by monoterpene hydrocarbons (29.87 %) and oxygenated monoterpenes (18.64 %).

Figure 2. Groups of components in dill seed oils, %:
1 – monoterpene hydrocarbons; 2 – oxygenated monoterpenes; 3 – phenyl propanoids; 4 – aliphatic hydrocarbons; 5 – oxygenated aliphatic.

The same dependence is observed in the seed oils. Phenyl propanoids (49.41 %) are the dominant group in the seed oil (2018), followed by oxygenated monoterpenes (14.18 %), oxygenated aliphatic (10.29 %), monoterpene hydrocarbons (8.17 %) and aliphatic hydrocarbons (1.30 %). In the seed oil after storage dominant group again are phenyl propanoids (56.62 %), followed by monoterpene hydrocarbons (21.77 %) and oxygenated monoterpenes (19.38 %). Phenyl propanoids (58.77 %) are the dominant group and in the seed oil from 2020, followed by monoterpene hydrocarbons (24.42 %) and oxygenated monoterpenes (15.25 %).
The group of phenyl propanoids, represented by methyl chavicol (32.90–62.96 %) dominates in all studied oils.

4. Conclusion
Bulgarian dill (Anethum graveolens L.) in the region of Gavrailovo village (42° 38' N и 26° 11' E), Sliven, Bulgaria is rich in essential oil: 2.21 – 4.40 % weed oil and 8.47 – 9.99 % seed oil respectively. A significant part of the content of essential oil in the herb (64.25 %) and fruits (77.88 %) was preserved during the storage of the raw material for a period of two years.

The presented data on chemical composition of dill essential oils during different harvest years and after storage showed that the group of phenyl propanoids, represented by methyl chavicol (32.90 – 62.96 %) dominates in all studied oils. The dominant component of the oils is methyl chavicol, which is the reason to be considered as a new methyl chavicol chemotype.

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