Chemical composition of Bulgarian white oregano (Origanum heracleoticum L.) essential oils

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Abstract. The content and composition of essential oils obtained from wild and cultivated Bulgarian white oregano (Origanum heracleoticum L.) were studied. Herb, inflorescences and leaves (2017 and 2018 harvest years) of wild oregano and leaves (2018 harvest year) of cultivated white oregano were examined. The influence of technological parameters on the yield of essential oil is studied. Content of the essential oils varies from 4,77% to 5,09% for the wild oregano and 5,74% for the cultivated. Aromatic compounds are in the highest amounts in all oils (64,63 – 86,16%). The main compounds (over 3%) in the wild white oregano essential oils were: carvacrol (57,52 – 75,29%), p-cymene (7,79 – 19,10%) and γ-terpinene (4,95 – 11,26%). The main compounds (over 3%) in the cultivated white oregano oil were: carvacrol (45,09%), thymol (12,06%), p-cymene (7,39%), γ-terpinene (5,20%) and thymol methyl ether (3,00%). The results supported the assumption that the studied overground parts of the Bulgarian white oregano are rich in essential oil containing the valuable aromatic compounds carvacrol and thymol, which are the reason for its properties.

1. Introduction
Oregano has been known since antiquity not only as a spice but also as a remedy that improves the health of the human body. The plant is rich in minerals, vitamins, fibers, tanning substances and other biologically active components with proven healing properties [1, 2, 3].

The white oregano (Origanum vulgare subsp. Hirtum (Link) Jeltswaart), (Syn. O. heracleoticum L., O. hirtum L.) is an annual herbaceous plant in the Lamiaceae family. It comes from the Mediterranean and is mainly cultivated in Greece, Italy and Spain. In Bulgaria white oregano grows naturally only in the southernmost parts of the country - the eastern slopes of the Rhodopes, Belasitsa, Strumsko valley and the Kresna gorge at an altitude of 250 to 700 m [4].

The main component of white oregano is the essential oil obtained mainly by hydrodistillation of pre-processed raw material [5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16]. The content of essential oil in white oregano varies from 1,1 % to 8,2 % [5, 8, 9, 11, 15, 16].

The chemical composition of the essential oil varies widely depending on a number of genetic, geographic, agro-meteorological and technological factors. Despite the botanical differences of the used raw material, the summarized results show that the obtained essential oils are characterized by the content of carvacrol (2,30 – 95,00%), thymol (0,20 – 90,20%), p-cymene (1,88 - 15,80%) and γ-terpinene (0,10 – 24,45%) as basic compounds [5, 6, 7, 8, 13, 14, 15, 16, 17, 18].
The essential oil of oregano has proven soothing, analgesic, spasmyolytic, antiseptic, antiparasitic and other healing properties, also it’s a suitable treatment for insomnia and stressful conditions [19]. Due to its proven antimicrobial and antioxidant properties according to the contained aromatic compounds of carvacrol and thymol, the white oregano essential oil can be used as a natural preservative in the food industry [12, 20, 21].

The data on the chemical composition of Bulgarian white oregano in the literature is very scarce. The aim of the present study is to enrich the information on its chemical composition as well as to track the influence of technological parameters on the production of essential oil. This will allow the medicinal plant and its aromatic products to be used as a natural component in the food, pharmaceutical and cosmetic industries.

2. Materials and Methods

2.1. Plant material

The subject of this study is the Bulgarian white oregano (Origanum heracleoticum L.): wild, harvested in its blooming phase in the southern slopes of the Eastern Rhodopes (360 m altitude), the region of Ivaylovgrad, Haskovo, Bulgaria at the end of July 2017 and 2018 years of harvest (pg). The dried overground part of the plant has been studied. The raw material is dried at room temperature without direct sunlight until moisture content reaches less than 10%. The percentage ratio between the individual plant parts is determined as follows: 31,50 % inflorescences, 36,20 % leaves, 32,50 % stems for 2017 harvest year and 40,80 % inflorescences, 31,10 % leaves, 28,10 % stems for 2018 harvest year; cultivated (leaves), in the territory of the town of Parvomay, Plovdiv, Bulgaria, harvest year 2018, commercially available.

In determining the authenticity according to the morphological features of the wild white oregano, in the Bulgarian flora, the plant is referred to as an inland taxon of the species Origanum vulgare L. in the genus Origanum (family Lamiaceae). The Flora of PR Bulgaria [4] was used for taxonomy.

2.2. Chemical analyses

The raw materials (air-dried) were analyzed for moisture content, by azeotropic distillation in a laboratory of Dyn and Stark, % [22]; for protein content, by Kjeldahl, % (BDS - ISO 5983-1: 2006); for fat content, by Soxlet, % (BDS - ISO 6492: 2007); for fiber content, by Henberg and Stoman, % (BDS - AOAC, 2007) and for ash content, by incineration in a muffle furnace at 650 °C, % (BDS - ISO 5984: 2007).

2.3. 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. 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 [22]. Prior to the technological processing the raw material has been cut into pieces measuring 1,5 - 2 cm [23].

Essential oils from the different parts of the plant (herb, inflorescences and leaves) at three hydromodules (HM): 1:20, 1:25 and 1:30 were obtained. The effect of the pre-soaking (hydration) of the raw material in a water with different temperature for 60 min on the yield of essential oil is monitored. The obtained essential oils are dehydrated with anhydrous Na2SO4 and stored in glass vials at 4 - 6 °C until analyzed. The yields of the essential oils are converted to an absolutely dry mass.

2.4. 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.

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 α=0,05.

3. Results and discussion

The composition of the air-dried white oregano – wild (2017 and 2018 harvest years) and cultivated (2018 harvest year) is presented in Table 1.

| Components     | Wild, 2017 harvest year | Wild, 2018 harvest year | Cultivated |
|----------------|-------------------------|-------------------------|------------|
| Moisture, %    | 7.98 ± 0.09             | 6.65 ± 0.06             | 5.98 ± 0.03|
| Proteins, %    | 7.18 ± 0.04             | 7.16 ± 0.05             | 12.56 ± 0.07|
| Fats, %        | 1.95 ± 0.08             | 3.07 ± 0.07             | 3.96 ± 0.08|
| Fibers, %      | 26.76 ± 0.07            | 14.13 ± 0.06            | 10.36 ± 0.09|
| Ash, %         | 7.11 ± 0.09             | 7.43 ± 0.08             | 8.90 ± 0.04|
| Essential oil, % w/v | 4.77 ± 0.05             | 5.09 ± 0.05             | 5.74 ± 0.05|

The protein content in the studied raw materials is comparable to the protein content of other Lamiaceae herbs - ordinary oregano, mint, thyme, sage, savory, rosemary, basil (4.88 – 22.98 %). The fat and fiber content is lower than in the raw materials compared (from 4.07 to 15.22 % for fats and from 37.00 to 45.70 % for fiber contents) because of their belonging to different botanical categories [3].

The yield of essential oil from Bulgarian wild and cultivated white oregano is comparable with the data in the literature – 1.1 to 8.2 % [5, 6, 7, 8, 9, 10, 11, 15, 17]. There is no significant difference between the contents of essential oil during the different harvest years. Cultivated white oregano is richer in essential oil than the wild one[10, 11, 16].

The content of essential oil in the various parts of the wild (herb, inflorescence, leaves for 2017 and 2018 harvest years) and the cultivated white oregano (leaves, 2018 harvest year) is shown in Figure 1.

The richest in essential oil is the inflorescence, which is comparable to literature data [24, 25]. There were no significant differences in the content of essential oil in inflorescences and leaves of the wild white oregano for the two harvest years. The amount of essential oil in the leaves of cultivated white oregano is higher than that in the leaves of the wild, which is the basis for its agricultural cultivation.

The influence of the hydromodule and the preliminary hydration of the raw material on the yield of essential oil and the duration of the distillation is shown in Figure 2 and Figure 3.
Figure 1. Essential oil content in different parts of the wild and cultivated white oregano.

Figure 2. Influence of the technological parameters on the yield of essential oil of wild white oregano – 2017 harvest year.

Figure 3. Influence of the technological parameters on the yield of essential oil of wild white oregano - 2018 harvest year.
The pre-hydration of the raw material for 60 min in water leads to the release of a higher amount of essential oil during the first 10 min (67.38 % at 20 °C and 72.90 % at 80 °C) and to shortening of the distillation process (at 30 min). The highest yield was recorded in a 1:25 hydromodule and during pre-hydration of the raw material for 60 min in 80 °C water.

The same effect of the technological parameters on the duration of distillation and the yield of essential oil was also observed with the cultivated white oregano.

The chemical composition of the essential oils from the different parts of wild white oregano for the two harvest years is presented in Table 2.

Table 2. Chemical composition of essential oils from the different parts of wild white oregano.

| №  | Components         | RI* | Herb oil Content, % (2017) | Flower oil Content, % (2017) | Leaf oil Content, % (2017) | Herb oil Content, % (2018) | Flower oil Content, % (2018) | Leaf oil Content, % (2018) |
|----|-------------------|-----|---------------------------|-----------------------------|---------------------------|---------------------------|-----------------------------|---------------------------|
| 1  | α-Thujene         | 908 | 0.89                      | 0.74                        | 0.68                      | 0.19                      | 0.72                        | 0.17                      |
| 2  | α-Pinene          | 929 | 0.65                      | 0.54                        | 0.47                      | 0.31                      | 0.53                        | 0.38                      |
| 3  | Sabinene          | 973 | 0.19                      | 0.15                        | 0.11                      | 0.12                      | 0.15                        | 0.21                      |
| 4  | β-Pinene          | 979 | 0.27                      | 0.23                        | 0.21                      | 0.20                      | 0.22                        | 0.17                      |
| 5  | 1-Octen-3-ol      | 980 | 0.64                      | 0.54                        | 0.47                      | nd**                      | nd                         | nd                       |
| 6  | 3-Octanone        | 985 | 0.15                      | 0.13                        | 0.13                      | nd                        | nd                         | nd                       |
| 7  | β-Myrcene         | 989 | 0.34                      | 0.28                        | 0.30                      | 1.19                      | 0.27                        | 0.75                      |
| 8  | 3-Octanol         | 998 | 0.12                      | 0.10                        | 0.11                      | nd                        | nd                         | nd                       |
| 9  | α-Terpinene       | 1018| 0.71                      | 0.59                        | 0.63                      | 1.35                      | 0.58                        | 0.79                      |
| 10 | p-Cymene          | 1026| 11.53                     | 19.10                       | 17.14                     | 7.79                      | 9.53                        | 18.11                     |
| 11 | Limonene          | 1030| 0.32                      | 0.27                        | 0.28                      | 0.78                      | 0.26                        | 0.36                      |
| 12 | 1,8-Cineole       | 1034| 0.30                      | 0.25                        | 0.27                      | 0.86                      | 0.25                        | 0.49                      |
| 13 | γ-Terpinene       | 1059| 0.32                      | 0.27                        | 0.28                      | 4.95                      | 11.26                       | 5.37                      |
| 14 | β-Linalool        | 1101| 0.31                      | 0.26                        | 0.27                      | 0.88                      | 0.25                        | 0.29                      |
| 15 | α-Thujone         | 1109| 0.12                      | 0.10                        | 0.30                      | 0.33                      | 0.19                        | 0.32                      |
| 16 | β-Thujone         | 1120| 0.10                      | 0.08                        | 0.19                      | 0.22                      | 0.28                        | 0.27                      |
| 17 | Borneol           | 1176| 0.48                      | 0.40                        | 0.42                      | 0.32                      | 0.39                        | 0.14                      |
| 18 | p-Cymen-8-ol      | 1179| nd                        | nd                         | nd                        | 0.17                      | nd                         | 0.54                      |
| 19 | Terpinen-4-ol     | 1183| 0.94                      | 0.78                        | 0.93                      | 0.67                      | 0.76                        | 0.35                      |
| 20 | α-Terpineol       | 1198| 0.18                      | 0.15                        | 0.56                      | 0.37                      | 0.14                        | 0.32                      |
| 21 | Thymol methyl ether | 1230| 0.89                      | 0.74                        | 0.79                      | 0.62                      | 0.46                        | 0.23                      |
| 22 | Carvacrol         | 1277| 68.77                     | 58.31                       | 57.52                     | 75.29                     | 70.85                       | 68.05                     |
| 23 | β-Caryophyllene   | 1426| 1.50                      | 1.25                        | 1.32                      | 1.21                      | 1.22                        | 0.26                      |
| 24 | α-Humulene        | 1462| 0.68                      | 0.57                        | 0.60                      | 0.47                      | 0.55                        | 0.17                      |
| 25 | γ-Muurolene       | 1483| 0.37                      | 0.31                        | 0.33                      | nd                        | nd                         | nd                       |
| 26 | Myristicin        | 1502| 1.27                      | 1.06                        | 1.11                      | nd                        | nd                         | nd                       |
| 27 | β-Bisabolene      | 1510| 2.31                      | 1.93                        | 2.04                      | nd                        | nd                         | nd                       |
| 28 | γ-Cadinene        | 1519| 0.81                      | 0.68                        | 0.71                      | nd                        | nd                         | nd                       |
| 29 | δ-Cadinene        | 1523| 0.34                      | 0.28                        | 0.30                      | nd                        | nd                         | nd                       |
| 30 | Caryophyllene oxide | 1590| 2.19                      | 1.83                        | 1.93                      | nd                        | nd                         | nd                       |
| 31 | Retinoic acid     | 2348| 2.13                      | 1.78                        | 1.88                      | nd                        | nd                         | nd                       |

RI* - Relative Index; nd** - the component is not identified
Thirty one compounds have been identified in the wild white oregano oil (2017 harvest year), representing 99.82 % of the total content identified in the herb oil, 93.70 % in the flower oil, and 92.28 % in the leaf oil.

Twenty-two compounds representing 98.76 % of the total oil content in the herb oil, 98.86 % - in the flower oil and 98.07 % - in the leaf oil, have been identified in the essential oils from the wild white oregano 2018 harvest year.

As seen the major constituents of the oils are carvacrol (57.52 – 75.29 %) and p-cymene (7.79 – 19.10 %). There was a higher content of γ-terpinene in the oils from 2018, which is probably due to differences in climatic conditions.

The distribution of aromatic components in the essential oils from the wild oregano, based on functional groups, showed that the aromatic compounds are the dominant group in the essential oils from all part of the wild plant and from both harvest years: 84.93 – 86.62 % in the herb oil, 81.77 – 84.54 % in the flower oil and 82.96 – 88.64 % in the leaf oil.

Because of the high content of carvacrol, the wild white oregano can be referred to as a mixed carvacrol chemotype.

Table 3 represents the chemical composition of the essential oil of the leaves of the cultivated white oregano and Figure 4 – the distribution of aromatic components based on functional groups.

| №  | Components          | RI*  | Content, % | №  | Components          | RI*  | Content, % |
|----|--------------------|------|------------|----|--------------------|------|------------|
| 1  | α-Thujene          | 924  | 0,83       | 24 | Terpinen-4-ol      | 1177 | 1,12       |
| 2  | α-Pinene           | 932  | 0,76       | 25 | α-Terpineol        | 1188 | 0,11       |
| 3  | β-Thujene          | 938  | 0,18       | 26 | Methyl nonanoate   | 1223 | 0,08       |
| 4  | Camphene           | 946  | 0,88       | 27 | Thymol methyl ether | 1230 | 3,00       |
| 5  | Sabinene           | 969  | 0,56       | 28 | Carvone            | 1241 | 0,14       |
| 6  | β-pinene           | 973  | 0,47       | 29 | Thymol             | 1289 | 12,06      |
| 7  | 1-Octen-3-ol       | 975  | 0,86       | 30 | Carvacrol          | 1298 | 45,09      |
| 8  | 3-Octanone         | 979  | 0,25       | 31 | Carvacrol acetate  | 1369 | 0,16       |
| 9  | Myrcene            | 988  | 1,06       | 32 | α-Copaene          | 1374 | 0,09       |
| 10 | α-Phellandrene     | 1002 | 0,65       | 33 | β-Bourbonene       | 1387 | 0,32       |
| 11 | 2E-Hexenoic acid   | 1005 | 0,26       | 34 | β-Caryophyllene    | 1418 | 2,24       |
| 12 | α-Terpinepine      | 1014 | 1,31       | 35 | β-Humulene         | 1435 | 0,19       |
| 13 | p-Cymene           | 1020 | 0,73       | 36 | α-Humulene         | 1452 | 0,63       |
| 14 | Limonene           | 1025 | 0,65       | 37 | γ-Muurolene        | 1478 | 0,27       |
| 15 | β-Phellandrene     | 1026 | 0,63       | 38 | trans-Muurola-4(14),5-diene | 1491 | 1,67 |
| 16 | β-cis-Ocimene      | 1032 | 1,39       | 39 | γ-Cadinen          | 1513 | 0,20       |
| 17 | β-trans-Ocimene    | 1043 | 0,58       | 40 | δ-Cadinen          | 1520 | 0,15       |
| 18 | γ-Terpinepine      | 1054 | 5,20       | 41 | (E)-γ-Bisabolene   | 1529 | 1,77       |
| 19 | n-Octanol          | 1062 | 0,54       | 42 | α-Cadinen          | 1537 | 0,21       |
| 20 | Terpinolene        | 1086 | 0,28       | 43 | Elemol             | 1548 | 0,38       |
| 21 | p-cymene           | 1090 | 0,10       | 44 | Caryophyllene oxide | 1583 | 1,53      |
| 22 | β-Linalool         | 1095 | 0,69       | 45 | n-Octadecane       | 1801 | 0,13       |
| 23 | Borneol            | 1164 | 1,48       | 46 | n-Heneicosane      | 2100 | 0,16       |

RI* - Relative Index
In the essential oil of cultured white oregano, forty-six compounds have been identified, which are 98.65% of the total composition.

As well as in the oils of wild oregano, aromatic compounds predominate (68.69%), represented by carvacrol (45.09%), thymol (12.06%) and p-cymene (7.39%). The composition of the essential oil is supplemented by monoterpenes (15.63%), their oxygen derivatives (3.59%), sesquiterpenes (7.84%), their oxygen derivatives (1.94%) and aliphatic compounds (2.31%).

The essential oil obtained from the air-dried Bulgarian white oregano in its blooming phase is an easily movable liquid with pale yellow, yellow-orange or red-brown color, with a fresh, spicy odor and a specific, pleasant hot, bitter-spicy flavor.

4. Conclusion
Bulgarian white oregano (Origanum heracleoticum L.) is rich in biologically active substances: proteins (7.16 – 7.18%); fats (1.95 – 3.07%); fibers (14.13 – 26.76%); essential oil (4.77 – 5.09%). The rich chemical composition of white oregano is the reason why the plant should be used not only as a spice but also as a raw material for the production of aromatic products.

The highest yield of essential oil was obtained in a 1:25 hydromodule and by pre-soaking of the raw material in hot water for 60 min. Predominant in the oil composition are the aromatic components represented by carvacrol (45.09 – 75.29%), thymol (12.06%) and p-cymene (7.39 – 19.10%), which are the reason for the properties of the plant. The higher content of essential oil in the cultivated white oregano (5.74%) than in the wild (4.77 – 5.09%) is a basis for the agricultural growing of the plant.

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