THE INFLUENCE OF THE DISTILLATION METHOD ON THE CHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF SAGE (*Salvia officinalis* L.)

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The chemical composition of the essential oils of sage (*Salvia officinalis* L.) has been investigated by GC-MS technique. Three ways of distillation, using a Clevenger-type apparatus were applied for their obtaining: I - classical hydrodistillation (CD), II - the 800W microwave hydrodistillation (MWD) and III - solvent free microwave distillation at 800W (SFMWD). Using the microwave, the distillation time was shortened to 75 min. (MWD) and 64 min. (SFMWD). As a characteristic component of the essential oil of sage, *cis*-thujone is mostly present in CD oil (19.1 %). A slightly lower content (18.8 %) was found in the SFMWD oil, while the lowest content (18.0 %) was observed in the MWD sample. The highest content of monoterpenes was identified in the MWD oil, followed by CD and SFMWD oils. Sesquiterpenes dominate in the SFMWD oil, followed by CD and MWD. The content of diterpenes (only manool) is the largest in the sample CD, followed by SFMWD and MWD.

Keywords: *Salvia officinalis*, microwave, distillation, chemical composition

Introduction

Bioactive products of natural origin are widely used in food and cosmetic industries, drug production, or as precursors in the synthesis of new products with specific bioactive properties. The production of high quality herbal extracts is the basis for the production of herbal preparations and the isolation of bioactive components that are widely used in industry [1]. Classical techniques have been mainly used for their obtaining, solvent extraction (maceration, percolation, the extraction with hot and cold fat and boiling in water), pressing or squeezing although, in recent years, newer non-conventional techniques have been increasingly used such as super- and sub-critical extraction [2], turbo extraction [3] or the extraction under the influence of ultrasound [4,5] and microwaves [6].

Microwaves have already found the application in the extraction of bioactive substances that are of interest for food and pharmaceutical industries, for example from medicinal plants [7,8] or fruits [9,10] and others. Also, under laboratory conditions microwaves are applied to isolate the essential oils of aromatic plants [11-14].

Sage (*Salvia officinalis* L.) has found a wide application in traditional medicine [15] and food industry in the form of various medicinal preparations, tea mixtures and culinary herbs and spices [16]. The available literature describes the chemical composition of the sage essential oil obtained by conventional distillation using a Clevenger-type apparatus [17, 18]. Since there are no data available on the possible influence of a distillation method on the chemical composition of the essential oil of sage, the aim of this paper was to examine the composition of oils obtained using three types of distillation: classical hydrodistillation (CD), the 800W microwave hydrodistillation (MWD) and the solvent free microwave distillation at 800W (SFMWD).

Experimental

Collection of the plant material. The herb of S. *officinalis* used for our study was collected in the Southeast Serbia in May 2011, in the stage of flowering. The plant material was collected at the locality Gradište village (Sićevačka klisura, the gorge in the surrounding of Niš). The voucher specimen was deposited at the Herbarium of the Insti-

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tute of Botany and Botanical Garden „Jevremovac“ of the Faculty of Biology, University of Belgrade (BEOU), under number 32147. The herb was air-dried immediately after harvesting in a shady site for 15 days, packed in paper bags and kept in a dark, dry and cool place. One part of the fresh herb was immediately used for chemical analysis. Moisture contents of dry and fresh herbal materials, determined by distillation with toluene [19], were 10.7 % (w/w) and 69.5 % (w/w) respectively.

Isolation of the essential oil. The plant materials were milled to a particle size of around 6 mm. Hydrodistillation processes were performed using a Clevenger-type apparatus [19]. The oils were stored for a few hours at 4 °C in the dark until tested and analysed.

Classical hydrodistillation

Dried chopped up plant material (100 g) was transferred into the 2 l balloon with a flat bottom. Into the same balloon, 1 l of tap water was poured so that the herb-to-water ratio was 1: 10 (w/V). The balloon was connected to the Clevenger-type apparatus for distillation. Warming was performed by a laboratory heating jacket (HedaS, Model 2000, 220V, 50Hz, 500W).

Microwave hydrodistillation

For this type of distillation the same apparatus and conditions were used as for the classical hydrodistillation.

Warming was performed by a modified microwave (Samsung M1712N, 800W, 230V, 50Hz, 2450MHz).

Solvent- free microwave distillation

Raw chopped up plant material (200 g) was transferred into the 2 l balloon with a flat bottom, without adding water. Warming was performed by the above mentioned microwave, power of 800 W.

Analysis of the essential oil. The oils were analyzed by analytical GC-FID and GC-MS using the normalization procedure, based upon the integration of chromatogram obtained by GC-FID.

The GC-FID analyses were carried out on a Hewlett Packard 5890 II Gas Chromatograph equipped with the HP-5 fused silica capillary column (25 m × 0.32 mm; 0.52 μm film thickness) and FID. GC oven temperature was linearly programmed from 40 °C to 280 °C at the rate of 4 °C/min and kept at 280 °C for 10 min. The injector was set to 250 °C and the detector to 280 °C. The carrier gas was H_2 at a flow rate of 1 mL/min. The essential oils (1 μL of 1 % V/V solution in absolute ethanol) were injected in a split mode (split ratio was 1:30).

The GC-MS analyses were performed on a Hewlett Packard G 1800 C system, using the HP-5MS fused silica capillary column (30 m × 0.25 mm; 0.25 μm film thickness), with He as the carrier gas at the flow rate of 1 mL/min. The temperature program, injected volume and split ratio were the same as for the analytical GC-FID. Electron Ionization mass spectra were acquired in scan mode in the m/z range 40 to 450.

Identification and quantification of oil constituents

The constituents were identified by comparing their mass spectra with those stored in MS libraries (Wiley 275, NIST05 and Adams2007), using different search engines (PBM, NIST 2.0), as well as calibrated AMDIS (ver. 2.64) for the determination and comparison of retention indices [20]. The quantification of essential oil components was achieved by the normalization method based upon the area percent report obtained by GC-FID.

Results and Discussion

The main objective of the study was to determine the chemical composition of the essential oils of S. *officinalis* obtained by the microwave power of 800W (MWD and SFMWD). The results were compared with CD where the oil yield was 1.35 ml/100 g of dry plant material. The duration of the conventional distillation amounted to 150 min., while when using the microwave the time was shortened to 75 min. and 64 min. (MWD and SFMWD, respectively; the total oil yields were 1.35 ml/100 g). The mechanism and kinetics of distillation of the essential oils obtained by CD and MWD have been explained in the paper [21].

The investigated oil of S. *officinalis* [18] contains all the characteristic components. The total participation of the characteristic monoterpenic components is 51.5 %: β-pinene (3.9 %), camphene (2.0 %), limonene (0.8), 1,8-cineole (16.2 %), linalool (0.4), *cis*-thujone (21.5 %), *trans*-thujone (2.7 %), camphor (4.0 %) and bornyl acetate (tr.). The mass percent of sesquiterpenes is 29.6. α-Humulene, a characteristic component, constitutes 11.2 %. Besides the mentioned 10 components, a large amount of β-pinene (7.6 %), viridiflorol (6.0 %), (*E*)-caryophyllene (5.0 %), borneol (3.2 %) and diterpene manool (2.2 %) were found.

The compositions of tested samples of S*. officinalis* essential oils are given in Table 1. The number of registered components in essential oils was 44, 39 and 33, while 38, 37 and 31 components were identified (CD, MWD and SFMWD, respectively). As a characteristic component of the essential oil of sage, *cis*-thujone is mostly present in CD oil (19.1 %). A slightly lower content (18.8 %) was found in the SFMWD oil, while the lowest content (18.0 %) was observed in the MWD sample. In the case of *trans*-thujone and camphor the differences were non-significant (MWD 2.4 %, CD 2.3 %, SFMWD 2.1 % for *trans*-thujone; MWD 2.9 %, CD 2.5 %, SFMWD 2.4 % for camphor). Within the MWD sample the content of α-pinene, camphene, β-pinene, 1,8-cineole, linalool, *trans*-thujone, and camphor prevailed. The SFMWD oil is characterized by a high content of limonene, borneol, α-humulene, (*E*)-caryophyllene, γ-muurolene, δ-cadinene, caryophyllene oxide, viridiflorol.

Table 1. Composition of *Salvia officinalis* essential oils

	CD			MWD			SFMWD		
Komponente	KIE	KIL	% m/m	KIE	KIL	% m/m	KIE	KIL	% m/m
(*Z*)-salvene	859.4	856	0.69	859.4	856	0.68	859.8	856	tr.
(*E*)-salvene	866.1	867	0.20	866.3	867	0.16	866.3	867	tr.
α-tujene	922.1	930	0.23	922.1	930	0.21	922.5	930	tr.
α-pinene	927.6	939	3.21	927.6	939	3.33	927.6	939	1.39
camphene	941.4	954	1.58	941.5	954	1.71	941.5	954	0.91
β-pinene	970.4	979	8.07	970.5	979	8.55	970.0	979	6.24
myrcene	988.4	991	0.73	988.5	991	0.73	989.3	991	0.51
p-cimene	1020.9	1025	0.23	1021.0	1025	0.23	1021.4	1025	tr.
limonene	1023.8	1029	tr.	n.c.	n.c.	n.c.	1024.4	1029	3.93
1,8-cineole	1027.2	1031	16.42	1027.5	1031	19.59	1026.5	1031	7.92
(*Z*)-β-ocimene	1040.0	1037	1.14	1035.2	1037	0.80	1035.6	1037	0.85
(*E*)-β-ocimene	1045.4	1050	0.43	1045.4	1050	0.43	1035.7	1050	tr.
γ-terpinene	1054.2	1060	0.53	1054.2	1060	0.51	1054.7	1060	tr.
terpinolene	1083.9	1089	0.15	1083.9	1089	tr.	n.c.	n.c.	n.c.
linalool	1101.5	1097	4.26	1101.5	1097	8.60	1102.0	1097	4.74
cis-thujone	1103.3	1102	19.14	1103.3	1102	18.00	1102.2	1102	18.82
n.i.	n.i.	n.i.	0.17	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
trans-thujone	1112.2	1114	2.26	1112.3	1114	2.40	1112.1	1114	2.10
n.i.	n.i.	n.i.	0.34	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
camphor	1138.6	1146	2.50	1138.6	1146	2.88	1138.5	1146	2.44
trans-pinocamphone	1156.1	1163	0.17	1156.2	1163	0.22	1156.2	1163	tr.
borneol	1161.5	1169	2.79	1161.6	1169	3.76	1161.3	1169	4.25
terpinen-4-ol	1174.2	1177	0.38	1174.4	1177	0.45	1174.4	1177	0.20
bornyl acetate	1281.2	1289	0.34	1281.1	1289	0.40	1281.3	1289	0.58
α-cubebene	1343.9	1351	0.22	1343.9	1351	0.18	1343.9	1351	0.33
α-ylangene	1367.0	1375	0.21	1365.1	1375	0.18	1365.1	1375	0.24
α-copaene	1369.5	1377	0.61	1369.5	1377	0.52	1369.5	1377	0.69
β-bourbonene	1378.4	1388	0.23	1378.4	1388	0.18	1378.2	1388	0.29
(*E*)-caryophyllene	1412.9	1419	5.73	1412.8	1419	5.22	1412.7	1419	9.82
β-copaene	1422.5	1432	0.34	1422.5	1432	0.29	1422.4	1432	0.66
α-humulene	1447.7	1455	8.72	1447.6	1455	7.61	1447.3	1455	11.61
allo-aromadendrene	1454.0	1460	0.11	1453.7	1460	0.10	1453.7	1460	0.20
γ-muurolene	1470.7	1480	1.38	1470.7	1480	1.13	1470.6	1480	1.75
viridiflorene	1488.7	1497	0.48	1488.7	1497	0.40	1488.6	1497	0.70
α-muurolene	1494.3	1500	0.28	1494.5	1500	0.23	1494.5	1500	0.39
γ-cadinene	1510.0	1514	0.58	1510.0	1514	0.51	1507.7	1514	0.91
δ-cadinene	1517.6	1523	1.63	1517.6	1523	1.31	1517.6	1523	2.27
caryophyllene oxide	1576.6	1583	0.66	1576.8	1583	0.49	1576.8	1583	0.83
viridiflorol	1586.5	1593	6.62	1586.2	1593	4.78	1585.9	1593	8.42
n.i.	n.i.	n.i.	0.16	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
humulene epoxide II	1602.8	1608	1.15	1602.9	1608	0.85	1602.8	1608	1.24
n.i.	n.i.	n.i.	0.26	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
n.i.	n.i.	n.i.	0.23	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
manool	2047.9	2057	4.23	2048.0	2057	1.58	2047.8	2057	4.18
n.i.	n.i.	n.i.	0.22	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
Monoterpene hydrocarbons	17.19			17.34			13.83		
Oxygenated monoterpenes	48.26			56.30			41.05		
Sesquiterpene hydrocarbons	20.52			17.86			29.86		
Oxygenated sesquiterpenes	8.43			6.12			10.49		
Oxygenated diterpenes	4.23			1.58			4.18		

CD - classical hydrodistillation
MWD - microwave hydrodistillation
SFMWD - solvent free microwave distillation
KIE - Kovats (retention) index experimentally determined by calibrated AMDIS (uncorrected values)
KIL - Kovats (retention) index - literature data [20]
% m/m - mass percent defined by peak area percent determined by integration (GC-FID)
tr. - traces (< 0.1 %)
n.i. - not identified
n.c. - not calculated

The highest content of monoterpenic hydrocarbons was identified in the MWD oil (17.3 %), followed by CD (17.2 %) and SFMWD (13.8 %) oils. It is similar with oxygenated monoterpenes as well (56.3 %, 48.3% and 41.1 %, respectively). Sesquiterpenic hydrocarbons dominate in SFMWD oil (29.9 %), followed by CD (20, 5 %) and MWD (17.9 %). Similarly, the content of oxygenated sesquiterpenes is the largest in the SFMWD sample (10.5 %), followed by CD (8.4 %) and MWD (6.1 %). The content of diterpenes (only manool) is the largest in the CD sample (4.2 %), followed by SFMWD (4.2 %) and MWD (1.6 %).

Summary

The chemical composition of the essential oils of sage (*Salvia officinalis* L.) has been investigated by GC-MS technique. Three ways of distillation using Clevenger-type apparatus were applied for their obtaining: I - classical hydrodistillation (CD), II - the 800W microwave hydrodistillation (MWD) and III – solvent-free microwave distillation at 800W (SFMWD). The oils were distilled from dried (for CD and MWD) and fresh (for SFMWD) herb. The duration of the conventional distillation amounted to 150 min. (I), while when using the microwave the time was shortened to 75 min. (II) and 64 min. (III). Based on the above, the third distillation that was carried out with the fresh plant material by microwaves at 800W, but without water in the system, was the fastest.

cis-Thujone was most present in the CD oil, obtained in a classical way which is used both in laboratory and in industry (19.1 %). The lowest content of *cis*-thujone (18.0 %) was found in the MWD sample, obtained by microwave hydrodistillation. Since the total thujone content is important regarding the safety of food and drinks, and its content slightly depends on the distillation technique, it can be concluded that the CD sample is the richest in total thujone (21.4 %). The SFMWD (20.9 %) and MWD (20.4 %) oils follow it.

The largest content of α-pinene, camphene, β-pinene, 1,8-cineole, linalool, *cis*-thujone, camphor and several other components was proven in the MWD oil. The SFMWD oil is characterized by a high content of limonene, borneol, α-humulene, (*E*)-caryophyllene, γ-muurolene, δ-cadinene, caryophyllene oxide, viridiflorol and other compounds.

The highest content of monoterpene hydrocarbons and oxygenated monoterpenes was identified in the MWD oil, followed by CD and SFMWD oils. Sesquiterpene hydrocarbons and oxygenated sesquiterpenes dominate within SFMWD oil, then CD and MWD. Diterpenes has been most found in the CD sample, and then SFMWD and MWD.

The above results indicate that the content of certain compounds in the essential oil of sage depends on the chosen technique of distillation.

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Abbreviations

CD	classical hydrodistillation
MWD	microwave hydrodistillation
SFMWD	solvent free microwave distillation
GC-MS	gas-chromatography / mass-spectrometry

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Izvod

UTICAJ METODE DESTILACIJE NA HEMIJSKI SASTAV ETARSKOG ULJA ŽALFIJE (*Salvia officinalis* L.)

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GC-MS tehnikom ispitan je hemijski etarskih ulja žalfije (*Salvia officinalis* L.). Za njihovo dobijanje korišćena su tri načina destilacije po Clevenger-u: I - klasična hidrodestilacija (CD), II - mikrotalasna hidrodestilacija na 800W (MWD) i III - suva mikrotalasna destilacija na 800W bez prisustva vode u sistemu (SFMWD). Vreme trajanja klasične destilacije je iznosilo 150 min. (I), dok je upotrebom mikrotalasa ovo vreme skraćeno na 75 min. (II) i 64 min. (III). Kao karakteristična komponenta etarskog ulja žalfije, *cis*-tujon je najviše prisutan u ulju CD (19,1 %). Nešto niži sadržaj (18,8 %) nađen je u ulju SFMWD, dok je najniži sadržaj (18,0 %) zabeležen u uzorku MWD. Najveći sadržaj monoterpena identifikovan je u ulju MWD, iza kog slede ulja CD i SFMWD. Seskviterpeni dominiraju u ulju SFMWD, zatim CD i MWD. Sadržaj diterpena (jedino manol) najveći je u uzorku CD, potom SFMWD i MWD.

Ključne reči: *Salvia officinalis*, mikrotalasi, destilacija, hemijski sastav