ON THE EFFICIENT EXTRACTION OF ESSENTIAL OIL FROM *MENTHA SPICATA*

Ioan CĂLINESCU[1], Adina Ionuță GAVRILĂ[2], Gabriel Călin IVOPOL[3], Maria IVOPOL[4], Nectara MIRCIOAGĂ[5], Mihaela BULEANDRĂ[6], Irinel Adriana BADEA[7], Mariana PĂTRAŞCU[8]

Essential oils and their components are especially important due to their utilisation in foodstuffs, cosmetics, pharmaceuticals and in medicine, being accepted as „safe" products by consumers. This paper's purpose was to obtain essential oil of spearmint (Mentha spicata L.) by microwave–assisted hydrodistillation. Hydrodistillation was carried out at atmospheric and reduced pressures, with dried spearmint, with or without enzymatic pretreatment. The effect of enzymatic pretreatment and pressure on the efficiency of the extraction process was observed. The analysis of the volatile components was carried out by GC-MS (the headspace method for the dried plant and liquid injection for the extracted essential oils).

Keywords: microwave-assisted hydrodistillation, *Mentha spicata*, headspace, essential oils

1. Introduction

The genus *Mentha* is arguably the most important in the Lamiaceae family due to its content in essential oils with varied applications in medicine, cosmetics and the food industry.

The Lamiaceae family comprises approximately 200 genera with between 2000 and 5000 species of plants. The genus *Mentha* comprises approximately 30 species from different regions of Europe, Asia, Australia and South America. The

[1] Prof., Bioresources and Polymer Science Department, University POLITEHNICA of Bucharest, Romania, e-mail: calin@tsocm.pub.ro
[2] Lecturer, Bioresources and Polymer Science Department, University POLITEHNICA of Bucharest, Romania, e-mail: adinagav@yahoo.com
[3] PhD student, Chemical and Biochemical Engineering Department, University POLITEHNICA of Bucharest, Romania
[4] PhD student, Chemical and Biochemical Engineering Department, University POLITEHNICA of Bucharest, Romania
[5] PhD student, Bioresources and Polymer Department, University POLITEHNICA of Bucharest, Romania
[6] Lecturer, Analytical Chemistry Department , University of Bucharest, Romania
[7] Prof., Analytical Chemistry Department, University of Bucharest, Romania
[8] PhD, SC PRIMOSAL SRL., Bucharest, Romania

classification of species from the genus *Mentha* is difficult due to the great variability in their morphological characteristics and ease of hybridisation, as is the case with *Mentha spicata*, which is the result of hybridising different species of *Mentha* [1-4].

Mentha spicata L. (spearmint) is an aromatic plant that is very appreciated in different areas. Spearmint contains compounds that confer it the following properties: antifungal, antiviral, antimicrobial, insecticidal, antioxidant, anti-allergic, diuretic and stimulating [5-8]. Both the leaves and essential oil have a stomachic, carminative, antiemetic, choleretic and mildly spasmolytic activity. It is used to treat fever, bronchitis, colds, cramps, gastritis, headaches, indigestion and nausea [6]. The leaves are used for tea and have a pleasant aroma. The essential oil has widespread use in the cosmetics industry (mouthwash, toothpaste, lozenges) as well as in the food industry. Menthol, the principal component, is used in medicine, cosmetics or the food industry [9].

By distilling the aboveground parts, an essential oil is obtained that contains a large number of aromatic chemical substances, such as menthol, menthone, isomenthone, menthofuran, carvone, linalool, and linalyl acetate that are used as aromas in foodstuffs and beverages, in the pharmaceuticals, cosmetics and associated industries [10-12].

Essential oils may be isolated, depending on the nature of the substrate, by: hydrodistillation (HD), dry distillation, extraction with solvents, hydrodiffusion, or by cold pressing [2]. The extraction method used for obtaining valuable compounds from natural raw materials has a bearing on product quality. Conventional extraction methods, such as extraction with organic solvents, pressing and maceration methods have a range of limitations. These limitations that reduce product quality or raise operating costs are: increased energy consumption, solvent use, elevated temperatures that can decompose thermolabile substances. For these reasons, lately the need has arisen to develop new extraction methods.

Data from scientific literature have shown that microwave-assisted extraction is a viable alternative to conventional methods due to a shorter extraction time, a more efficient and selective heating, reduced solvent consumption, simpler equipment, pollution prevention and a greater purity of the final product [13-17].

Another alternative method for improving the extraction of essential oils is enzymatic pretreatment, for hydrolysing the cellular membranes of plant vacuoles that contain essential oils. With regard to their specificity and high efficiency, enzymes are useful for extracting essential oils [18-20].

As a result, the aim of this paper was to obtain essential oil of spearmint (*Mentha spicata* L.) by microwave assisted hydrodistillation with or without enzymatic pretreatment and correlating the composition of the oil obtained with

headspace analysis of the dried plant. Hydrodistillation was carried out at atmospheric pressure and at reduced pressure, using dried spearmint with or without enzymatic pretreatment. The effect of the enzymatic pretreatment and pressure on extraction yield and chemical composition of the essential oils obtained was monitored. The analysis of the essential oil components was carried out by GC-MS (the headspace method for the dried plant and liquid injection for the extracted essential oils).

2. Experimental

Plant Material and Chemicals. The raw material, spearmint (*Mentha spicata* L.) was obtained from Hofigal S.A. in Bucharest. The plants were harvested in early May, before the flowering period. The plant was dried and dosed in 25 gram portions.

Cellulases (Carezyme 4500T, Celluclean 700T, Celluzyme 0.7 T) were purchased from Novozyme, Denmark. Di-sodium hydrogen phosphate 2-hydrate ($Na_2HPO_4*2H_2O$) was obtained from Merck and citric acid was purchased from Aldrich.

Enzymatic pre-treatment. Dried spearmint (25 g) was mixed with 500 mL pretreatment solution (200 mL of buffer solution and 300 mL of ultrapure water). The buffer mixture was obtained from two solutions: 0.2 M $Na_2HPO_4*2H_2O$ and 0.1 M citric acid to keep the correct pH = 4.6 for enzymes pretreatment. A mixture of enzymes was used: 0.1 g Carezyme 4500T, 0.1 g Celluclean 700T and 0.1 g Celluzyme 0.7 T). The mixtures were stirred for 1 hour at 40°C then subjected to microwave-assisted hydrodistillation (MWHD) for essential oil extraction. Experiments were realized under reduced (0.7 bar) or atmospheric pressure. Control samples of dried spearmint without any pretreatment were directly subjected to MWHD.

MWHD apparatus and procedure. The essential oils were extracted using microwave-assisted hydrodistillation using a multimode microwave reactor (MINILABOTRON 2000). The hydrodistillation (HD) was carried out by concurrently bubbling nitrogen gas at 5 L/h to avoid oxidation of the components. During the experiments, time, temperature, pressure and power were controlled from an operating console. Steam produced in the reactor carrying the spearmint essential oil was directed to a modified Clevenger trap with 5.00 mL graduated tube. The separated oil was analysed and kept at 4°C.

GC-MS analysis. The headspace GC-MS instrumentation consisted of the Thermo Electron system, provided with a Triplus HS Autosampler. The 20 mL headspace vial containing a quantity of 0.5 g of dry spearmint was heated to 80°C for 10 minutes and 500 μL of the headspace gas was injected into the column. The GC-MS analyses were performed with a Focus GC chromatograph coupled with a

Polaris Q ion trap mass detector. A DB-5MS capillary column (25 m x 0.25 mm; 0.25 μm of film thickness) was used, and the carrier gas was helium at 1 mL/min. The GC oven temperature program was: initial temperature 60°C (3 min) followed by an increase of 10°C /min up to 200°C (2 min) and then 12°C /min to the final temperature of 240°C (2 min). The source and interface temperature were 200°C and 250°C, respectively. Detector operated in electron impact mode (70 eV). Detection was performed in the range of m/z 35-300. The mass spectrometer was operated in the full scan mode. All peaks of the chromatograms were analyzed using Xcalibur® software and NIST 11 Mass Spectral Library in order to identify the corresponding compound. Alkane standard solution for GC (C8-C20 in hexane) was used for retention indexes (RI) calculation [7]. Relative percentages of the individual components were calculated based on GC peak areas.

For analysis of the essential oil, the samples were diluted with ultrapure hexane (1 to 10) and then amounts of 1 μL were injected.

2. Results and discussions

The volatile components from the *Mentha spicata L.* sample and from the essential oils obtained by microwave-assisted hydrodistillation were respectively analysed and identified by GC-MS. The results of the headspace (HS) analysis of dried spearmint and GC-MS analyses for the essential oils obtained from dried spearmint obtained under different pressure and enzymatic pretreatment regimes are presented in table 1.

Table 1

Compounds identified in the dried *Mentha spicata* sample (HS analysis) and in the essential oils obtained therefrom

RT	RI calc	Compound / CAS	Dried plant analysis (HS)		Essential oil analysis					
					Atmospheric Pressure		Reduced Pressure		With pretreatment	
			Area /10^8	%	Area /10^8	%	Area /10^8	%	Area /10^8	%
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*
4.36	861	Butanoic acid, 2-methyl-, ethyl ester 7452-79-1	2.8E-1	0.72	-	-	-	-	-	-
5.59	929	Santoline triene 2153-66-4	2.4E-1	0.62	5.3E-3	0.04	-	-	5.5E-3	0.04
5.72	936	α-Thujene 2867-05-2	2.3E-1	0.60	4.9E-3	0.04	2.1E-4	0.00	4.9E-3	0.03
5.87	945	α-Pinene 80-56-8	4.4	11.13	9.8E-2	0.76	2.6E-3	0.03	1.1E-1	0.72
6.14	959	Camphene 79-92-5	1.2E-1	0.30	2.7E-3	0.02	-	-	2.9E-3	0.02
6.58	983	Sabinene 3387-41-5	1.6	4.06	7.3E-2	0.57	2.9E-3	0.03	9.6E-2	0.65

6.67	988	β-Pinene 127-91-3	3.1	7.82	1.3E-1	1.01	5.4E-3	0.06	1.7E-1	1.14
6.85	998	β-Myrcene 123-35-3	3.9	9.97	1.7E-1	1.35	9.9E-3	0.11	2.2E-1	1.49
6.94	1003	3-Octanol 589-98-0	4.1E-2	0.11	9.5E-3	0.07	5.8E-3	0.06	9.8E-3	0.07
7.12	1013	α-Phellandrene 99-83-2	1.4E-1	0.35	-	-	-	-	-	-
7.23	1020	3-Carene 13466-78-9	4.6E-3	0.01	-	-	-	-	-	-
7.33	1026	α-Terpinene 99-86-5	1.6E-2	0.04	2.0E-3	0.02	4.8E-4	0.01	2.4E-3	0.02
7.60	1041	Limonene 138-86-3	1.1E+1	27.25	1.1	8.66	8.5E-2	0.93	1.5	10.00
7.66	1045	Eucalyptol (1,8-Cineole) 470-82-6	4.4	11.21	4.2E-1	3.27	9.4E-2	1.02	3.7E-1	2.49
7.84	1055	trans-β-Ocimene 3779-61-1	1.0E-1	0.26	6.4E-3	0.05	6.9E-4	0.01	8.0E-3	0.05
8.06	1068	γ-Terpinene 99-85-4	1.5E-2	0.04	3.4E-3	0.03	1.1E-3	0.01	4.3E-3	0.03
8.24	1078	trans-Sabinene hydrate 17699-16-0	6.6E-1	1.69	1.9E-1	1.50	1.6E-1	1.70	1.7E-1	1.12
8.35	1085	α-Pinene oxide 1686-14-2	4.3E-2	0.11	5.9E-3	0.05	1.9E-3	0.02	5.2E-3	0.03
8.55	1097	p-Cymenene 1195-32-0	-	-	-	-	1.9E-3	0.02	-	-
8.56	1097	α-Terpinolene 586-62-9	7.3E-2	0.19	1.0E-2	0.08	0.0		1.4E-2	0.09
8.71	1106	Linalool 78-70-6	2.0E-1	0.51	1.3E-2	0.10	1.2E-2	0.13	1.3E-2	0.09

1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*
8.78	1111	Isothujol 513-23-5	3.8E-2	0.10	1.2E-2	0.09	5.5E-3	0.06	1.4E-2	0.09
9.08	1130	trans-Pinocarveol 547-61-5	1.1E-2	0.03	1.4E-3	0.01	-	-	2.0E-3	0.01
9.12	1132	trans-p-Menth-2,8-dien-1-ol 7212-40-0	-	-	1.7E-3	0.01	1.4E-3	0.01	1.8E-3	0.01
9.34	1146	cis-Limonene oxide 13837-75-7	4.9E-3	0.01	9.0E-4	0.01	5.6E-04	0.01	6.6E-4	0.00
9.40	1150	trans-Limonene oxide 4959-35-7	2.4E-2	0.06	6.9E-3	0.05	4.2E-3	0.05	5.5E-3	0.04
9.57	1161	Camphor 76-22-2	5.0E-2	0.13	0.0		0.0		0.0	
9.64	1165	cis-p-Menth-2,8-dien-1-ol 3886-78-0	-	-	-	-	6.2E-4	0.01	9.8E-4	0.01
9.88	1180	Borneol 507-70-7	3.8E-2	0.10	-	-	-	-	-	-
10.04	1191	Terpinen-4-ol 562-74-3	2.1E-2	0.05	1.2E-2	0.09	1.0E-2	0.11	1.2E-2	0.08

10.25	1204	α-Terpineol 98-55-5	3.3E-2	0.09	3.2E-2	0.25	2.6E-2	0.28	3.3E-2	0.22
10.30	1208	Dihydro-carveol 619-01-2	2.2E-1	0.57	3.0E-1	2.33	2.3E-1	2.55	4.3E-1	2.88
10.36	1212	trans-Dihydro-carvone 5948-04-9	6.6E-1	1.68	3.7E-1	2.89	3.2E-1	3.51	3.9E-1	2.59
10.68	1234	trans-Carveol 1197-07-5	1.5E-2	0.04	4.1E-2	0.32	3.3E-2	0.37	8.2E-2	0.55
10.88	1247	cis-Carveol 1197-06-4	1.3E-2	0.03	3.3E-02	0.25	5.9E-2	0.64	6.9E-2	0.46
11.01	1256	Pulegone 89-82-7	3.1E-2	0.08	1.6E-2	0.13	1.6E-2	0.18	1.7E-2	0.11
11.13	1264	Carvone 99-49-0	7.3	18.70	9.3	72.55	7.7	84.19	1.0E+1	69.83
11.35	1279	Perilla aldehyde 2111-75-3	5.4E-3	0.01	8.5E-3	0.07	5.8E-3	0.06	1.1E-2	0.07
11.55	1293	cis-Carvone oxide 18383-49-8	9.1E-3	0.02	1.4E-2	0.11	9.4E-3	0.10	1.8E-2	0.12
11.71	1304	Dihydroedulan II 41678-32-4	-	-	-	-	-	-	1.1E-3	0.01
12.19	1340	Dihydrocarvyl acetate 20777-49-5	9.4E-2	0.24	8.1E-2	0.63	4.3E-2	0.47	1.4E-1	0.95
12.31	1349	trans-Carvyl acetate 97-42-7	-	-	1.9E-3	0.01	1.2E-3	0.01	2.9E-3	0.02
12.50	1363	Piperitenone 491-09-8	-	-	3.0E-3	0.02	3.2E-3	0.03	9.3E-3	0.06

1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*
12.66	1374	cis-Carvyl acetate 1205-42-1	4.6E-2	0.12	4.6E-2	0.36	2.5E-2	0.28	7.8E-2	0.52
12.91	1393	α-Copaene 3856-25-5	7.4E-3	0.02	5.2E-4	0.00	8.6E-4	0.01	1.3E-3	0.01
13.06	1404	β-Bourbonene 5208-59-3	1.4E-1	0.35	1.1E-2	0.09	2.1E-2	0.23	2.5E-2	0.17
13.10	1407	β-Elemene 515-13-9	4.1E-2	0.11	1.1E-2	0.08	9.4E-3	0.10	2.0E-2	0.14
13.22	1417	cis-Jasmone 488-10-8	0.0		7.4E-2	0.57	3.4E-2	0.37	1.1E-1	0.71
13.54	1442	β-caryophyllene 87-44-5	9.3E-2	0.24	9.7E-3	0.08	2.0E-2	0.22	2.9E-2	0.19
13.64	1450	Alloaromaden-drene 25246-27-9	1.9E-2	0.05	2.2E-3	0.02	3.4E-3	0.04	5.0E-3	0.03
13.84	1465	γ-Muurolene 30021-74-0	3.0E-2	0.08	1.2E-2	0.09	8.6E-3	0.09	2.0E-2	0.13
13.98	1476	α-Selinene 473-13-2	3.1E-3	0.01	4.7E-3	0.04	3.8E-3	0.04	5.4E-3	0.04
14.08	1484	Valencene 4630-07-3	2.6E-2	0.07	7.6E-3	0.06	1.0E-2	0.11	1.4E-2	0.10
14.14	1489	α-Muurolene 31983-22-9	6.9E-3	0.02	1.3E-3	0.01	2.3E-3	0.02	2.9E-3	0.02

14.32	1503	γ-Cadinene 39029-41-9	2.0E-1	0.51	5.5E-2	0.43	9.1E-2	1.00	1.1E-1	0.75
14.78	1542	δ-Cadinene 483-76-1	5.4E-4	0.00	0.0		0.0		0.0	
14.81	1545	cis-Calamenene 483-77-2	9.8E-3	0.03	6.0E-3	0.05	7.0E-3	0.08	1.1E-2	0.08
14.98	1559	β-Cadinene 523-47-7	1.9E-3	0.00	8.2E-4	0.01	1.1E-3	0.01	1.5E-3	0.01
15.21	1578	Caryophyllene oxide 1139-30-6	-	-	3.8E-4	0.00	2.1E-4	0.00	9.8E-4	0.01
15.52	1604	Spathulenol 77171-55-2	-	-	2.5E-3	0.02	6.3E-4	0.01	4.5E-3	0.03
15.73	1623	Globulol 51371-47-2	1.6E-3	0.00	1.4E-2	0.11	5.5E-3	0.06	2.2E-2	0.14
15.94	1642	Cubenol 21284-22-0	-	-	7.4E-3	0.06	2.6E-3	0.03	1.2E-2	0.08
16.22	1666	β-Eudesmol 473-15-4	-	-	3.0E-3	0.02	9.6E-4	0.01	4.1E-3	0.03
16.39	1681	α-Cadinol 481-34-5	-	-	2.3E-3	0.02	5.4E-4	0.01	3.9E-3	0.03

Around 70 compounds were identified by GC-MS analysis, of which carvone was the most abundent, followed by limonene, eucalyptol, β-myrcene, α and β-pinene.

From analysis of the data presented in Table 1 it can be seen that the majority of the components that were highlighted by the HS method are also found in the essential oil.

Next, a procedure shall be applied for quantifying the efficiency of the essential oil extraction taking the HS analysis as a reference point.

For each component, a normalised area of the plant mass used was calculated which also takes into consideration the mass of the processed plant, respectively the size of the sample obtained.

Normalised area for HS analysis:

$$A_{HS\_N} = \frac{A_{peak} * V_{vial} / V_{sample}}{m_1} \tag{1}$$

where:

A_{peak} is the peak area from the chromatogram

V_{vial} is the vial volume used in the HS analysis (20 mL);

V_{sample} is the volume of gas injected (0.5 mL)

m_1 is the mass of the analysed plant (0,5 g)

Normalised area for essential oil analysis:

$$A_{EO\_N} = \frac{A_{peak} * V_{EO} / V_{sample} * DF}{m_2} \tag{2}$$

where:

V_{EO} is the volume of essential oil obtained (see table 2);
V_{sample} is the volume injected (0.001 mL)
DF is the dilution factor in hexane (10)
m_2 is the plant mass subjected to extraction (25 g)

Microwave-assisted hydrodistillation of *Mentha spicata* was carried out in different conditions in order to determine the optimum efficiency of the extraction process. The essential oil was obtained at atmospheric and at reduced pressure (0.7 bar) without enzymatic pretreatment and after an enzymatic pretreatment. The results of the extraction of essential oil from *Mentha spicata* are presented in Table 2.

Table 2

Results of the extraction of essential oil from *Mentha spicata*

Exp. No.	Extraction conditions	Results	
		Extraction time, min	Volume extracted, mL
1	Without pretreatment, at atmospheric pressure	30	0.55
2	Without pretreatment, at reduced pressure	30	0.23
3	With pretreatment, at atmospheric pressure	20	0.58

From the analysis of the results presented in table 2, it can be seen that obtaining essential oil at reduced pressure did not lead to very good results, a substantial part of the compounds were not condensed in the refrigerant.

As for the enzymatic pretreatment, the extraction time was reduced and the volume of essential oil obtained rose slightly. This outcome may be explained by the modification of the structure of the cell wall due to hydrolysis resulting from the action of cellulases, which leads to an intensification of the extraction of essential oil.

By reporting the normalised areas of the main compounds identified in the essential oil to the normalised areas of the same components identified in the HS analysis, the values presented in Fig. 2 are obtained.

It can be seen that the quantities of the most volatile compounds extracted in the essential oil are less than those obtained in the HS analysis. This difference is greater proportionate to the increased volatility of the compounds.

By using enzymatic pretreatment, better ratios are obtained for the main analysed compounds, so that in this case the extraction efficiency is improved.

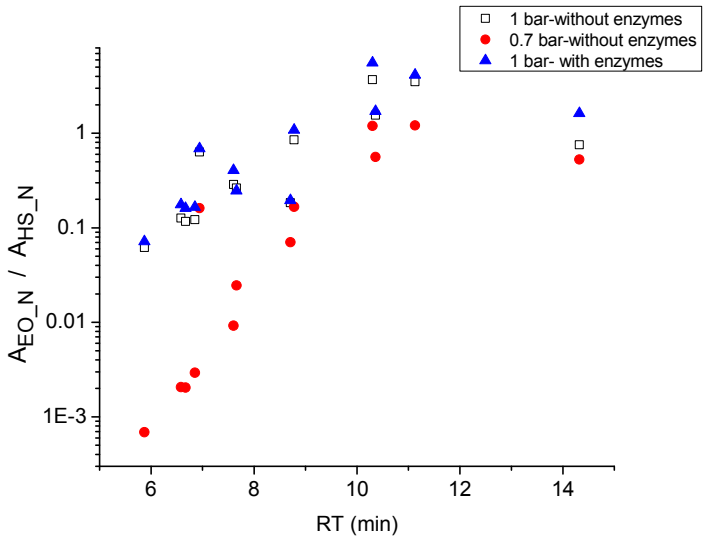

Fig. 2. The normalised area ratio for the main compounds identified from the essential oil extracted by different methods

Fig. 3 presents the concentrations of the main components from *Mentha spicata* (Headspace) and from essential oils (GC-MS) obtained in different conditions.

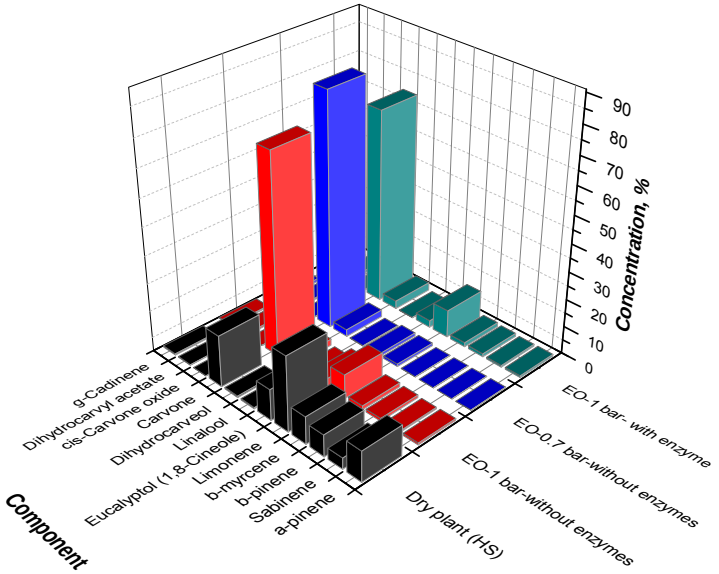

Fig. 3. Main components concentration for *Mentha spicata* (Headspace) and for essential oils (EO) obtained in different conditions

From the analysis of the results presented in Fig. 3, it can be seen that from the volatile compositional analysis of spearmint by Headspace, the more volatile compounds stand out, whose concentration in the sample is quite high. In the essential oil samples, the concentration of the more volatile compounds is much diminished, the main compound being carvone, this is obtained in the greatest proportion when extraction is performed at reduced pressure.

For the enzymatic pretreatment, the composition of the essential oil does not differ too much from that obtained in the absence of enzymatic pretreatment and atmospheric pressure.

4. Conclusions

Essential oil of spearmint was obtained by microwave-assisted hydrodistillation, at atmospheric and at reduced pressure, with or without enzymatic pretreatment. Volatile compounds from *Mentha spicata* were identified by GC-MS analysis. By comparing the Headspace analyses carried out on dried spearmint with the essential oil obtained from dried spearmint, the extraction efficiency of the main components was determined.

For extraction at reduced pressure, the extraction efficiency is lower when the volatile compound content of the sample is greater. For extraction with enzymatic pretreatment, the extraction efficiency is greater for the main compounds analysed.

Acknowledgments

Authors thank for financial support to Romania – Turkey Joint Project 604/2013 financed by ANCS.

R E F E R E N C E S

[1] *H. J. Dorman, M. Kosar, K. Kahlos, Y. Holm, R. Hiltunen,* "Antioxidant properties and composition of aqueous extracts from Mentha species, hybrids, varieties and cultivars", Journal of Agricultural and Food Chemistry, **vol. 51,** no. 16, 2003, p. 4563-4569.

[2] *P. P. Almeida, N. Mezzomo, S. R. S. Ferreira,* "Extraction of *Mentha spicata L*. Volatile Compounds: Evaluation of Process Parameters and Extract Composition", Food and Bioprocess Technology, **vol. 5**, 2012, p. 548–559.

[3] *R.S. Verma, L. Rahman, R.K. Verma, A. Chauhan, A.K. Yadav,* A. Singh, "Essential Oil Composition of Menthol Mint (*Mentha arvensis*) and Peppermint (*Mentha piperita*) Cultivars at Different Stages of Plant Growth from Kumaon Region of Western Himalaya", Journal of Medicinal and Aromatic Plants **vol. 1**, no. 1, 2010, p. 13-18. A

[4] *T. V. Khomova, S. D. Gusakova, and A. Nigmatullaev,* "Lipids of *Mentha spicata"*, Chemistry of Natural Compounds, **vol. 33**, no. 6, 1997, p. 630-632

[5] *S. Alankar,* "A review on peppermint oil" Asian Journal of Pharmaceutical and Clinical Research, **vol. 2**, Iss 2, April- June, 2009, p. 27-33.

[6] *A. E. Edris*, "Pharmaceutical and therapeutic Potentials of essential oils and their individual volatile constituents: a review", Phytotherapy Research, **vol. 21**, Iss. 4, 2007, p. 308–323.

[7] *R. P. Choudhury, A. Kumar, A. N. Garg,* "Analysis of Indian mint (Mentha spicata) for essential, trace and toxic elements and its antioxidant behavior" Journal of Pharmaceutical and Biomedical Analysis, **vol. 3**, no.7, 2006, p. 825–832.

[8] *S. R. Kanatt, R. Chander, A. Sharma,* "Antioxidant potential of mint (Mentha spicata L.) in radiation-processed lamb meat" Food Chemistry, vol. 100, no. 2, 2007, p. 451–458.

[9] *H. Hajlaoui, N. Trabelsi, E. Noumi, M. Snoussi, H. Fallah, R. Ksouri, A. Bakhrouf*, "Biological activities of the essential oils and methanol extract of tow cultivated mint species (*Mentha longifolia* and *Mentha pulegium*) used in the Tunisian folkloric medicine", World Journal of Microbiology and Biotechnology, **vol. 25**, 2009, p. 2227–2238.

[10] *J.R. Bahl, R.P. Bansal, S.N. Garg, A.A. Naqvi, R. Luthra, A.K. Kukreja, S. Kumar* "Qualitative evaluation of the essential oils of the prevalent cultivars of commercial mint species Mentha arvensis, M. spicata, M. pipermint, M. cardiaca, M. citrata and M. viridis cultivated in indo-gangetic plains", Journal of Medicinal and Aromatic Plants Sciences **vol. 22**, 2000, p. 787-797.

[11] *M. Bimakr, R.A. Rahman, F.S. Taip, L.T. Chuan, A. Ganjloo, L. Md Salleh, J. Selamat and A. Hamid*, "Supercritical Carbon Dioxide (SC-CO) Extraction of Catechin, Epicatechin, Rutin and Luteolin from Spearmint (*Mentha spicata L.*) Leaves", World Applied Sciences Journal vol. 5 No. 4, 2008, p. 410-417.

[12] *L. Rodrigues, A. Duarte, A. C. Figueiredo, L. Brito, G. Teixeira, M. Moldao, A. Monteiro,* "Chemical composition and antibacterial activity of the essential oils from the medicinal plant Mentha cervina L. grown in Portugal", Medicinal Chemistry Research **vol.21**, 2012 p. 3485–3490.

[13] *M. E. Lucchesi, F. Chemat, J. Smadja,* "Solvent-free microwave extraction of essential oil from aromatic herbs: comparison with conventional hydro-distillation". Journal of Chromatography. A, **vol. 1043**, 2004, p. 323–327.

[14] *N. Sahraoui, M. A. Vian, I. Bornard, C. Boutekedjiret, F. Chemat,* "Improved microwave steam distillation apparatus for isolation of essential oils Comparison with conventional steam distillation", Journal of Chromatography A, **vol. 1210,** 2008, p. 229–233.

[15] *F. Chemat and G.C. Cravotto,* "Microwave-assisted Extraction for Bioactive Compounds", Springer Science + Business Media, New York, 2013.

[16] *F. Chemat, M.E. Lucchesi, J. Smadja, L. Favretto, G. Colnaghi, F. Visinoni,* "Microwave accelerated steam distillation of essential oil from lavender: A rapid, clean and environmentally friendly approach" Analytica Chimica Acta, **vol. 555**, 2006, p. 157-160.

[17] *M. A. Vian, X. Fernandez, F. Visinoni, F. Chemat,* "Microwave hydrodiffusion and gravity, a new technique for extraction of essential oils", Journal of Chromatography A, **vol. 1190,** 2008, p. 14–17.

[18] *J. Li, Y. G. Zu, M. Luo , C. B. Gu, C. J. Zhao, T. Efferth, Y. J. Fu,* "Aqueous enzymatic process assisted by microwave extraction of oil from yellow horn (*Xanthoceras sorbifolia Bunge.*) seed kernels and its quality evaluation", Food Chemistry **vol. 138**, 2013, p. 2152–2158.

[19] *K. Hosni, I. Hassen, H. Chaâbane, M. Jemli, S. Dallali, H. Sebei, H. Casabianca*, "Enzyme-assisted extraction of essential oils from thyme (*Thymus capitatus L.*) and rosemary (*Rosmarinus officinalis L.*): Impact on yield, chemical composition and antimicrobial activity", Industrial Crops and Products **vol. 47**, 2013, p. 291– 299.

[20] *A. L. Groussin, S. Antoniotti,* "Valuable chemicals by the enzymatic modification of molecules of natural origin: Terpenoids, steroids, phenolics and related compounds", Bioresource Technology **vol. 115,** 2012, p. 237–243