THE CHROMATOGRAPHIC ANALYSIS OF CARAWAY ESSENTIAL OIL AS THE POTENTIAL BIOPESTICIDE

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Abstract
The chromatographic analysis of essential oil was carried out by recording the mass spectrums of the detected components by GC-MS. After confirming the components through retention times and Kovats indices, their quantification was done by GC-FID. The main constituents of the caraway essential oil are carvone with 68.22% and limonene with 21.80% in content. Beside carvone and limonene there were 25 constituents which in total make less than 10.00% of the studied essential oil.

Introduction

In recent years essential oils have drawn attention due to their biological effect as potential agents in pest control [1]. As the by-products of plant metabolism they are regarded as evaporable secondary metabolites of plants which are the mixture of mono and sesquiterpenes. The biological activity of essential oils depends on their chemical composition, the part of the plant they have been extracted from, phenological state of the plant, environmental conditions and the extraction methods [2].

Caraway (*Carum* carvi) is an annual or biannual herbaceous plant with an axial root system from *Apiaceae*family. The name of the genus is derived from the Greek*kar*or *kara*,which means „head“, due to the appearance of its inflorescence [3]. Most essential oils are active in low concentrations (0.1 g/kg of food). The chemical composition of the essential oil of C. carvi collected from various countries has been widely studied and great variations in essential oil content and chemical composition of the essential oil were observed. Many data indicated that the essential oil possessed antimicrobial, antifungal, molluscidal, nematicidal, antioxidant and antiaflatoxigenic activities, as well as potential as a cancer preventing agent [4].

The content of essential oils, the amount of carvone and limonene in the oil and the ratio of both substances are the main quality criteria determined in caraway production. To determine carvone and limonene contents, mostly the gas chromatography with flame ionisation (GC/FID) or mass spectrometry (GC/MS) detection are used. Highperformance liquid chromatography (HPLC) with polarimetric detection, derivative spectrophotometry and proton magnetic resonation can also be applied [5].

Since the main components of essential oils are considered responsible for their biological activity, the objective was to determine the chemical composition of caraway essential oil by the chromatographic analysis, obtained in the distillation by water vapor.

Experimental

The caraway essential oil was extracted from the fruit (fructus) by hydrodestilation (HD) with n-hexane as an organic solvent/recipient, collected on a private farm at Mošorin (N 45°17'28.113 E 20°11'43.80936). Thirty grams of caraway were subjected to hydrodestilation for 3 hours using a Clevenger-type apparatus. The essential oil was collected over water, separated, dried over anhydrous sodium sulphate, and stored in the dark at 4 ºC.

The chromatographic analysis of essential oil was carried out by recording the mass spectrums of the detected components by gas chromatography with mass spectrometry (GC-MS). After confirming the components through retention times and Kovats indices, their quantification was done by the gas chromatography with flame ionization detector (GC-FID).

GC/FID analysis of tested samples of essential oils was carried out on an Agilent Technologies, model 7890A gas chromatograph, equipped with split-splitless injector and automatic liquid sampler (ALS), attached to HP-5MS column (30 m x 0.25 mm, 0.25 µm film thickness) and fitted to flame ionisation detector (FID). Carrier gas flow rate (H2) was 1 ml/min, injector temperature was 250 °C, detector temperature 300 °C, while column temperature was linearly programmed from 40-260 °C (at the rate of 4 °C /min), and held isothermally at 260 °C next 5 minutes. Solutions of tested samples in EtOH (~15 ml/ml) were consecutively injected by ALS (2 µl, split mode 1:30). Area percent reports, obtained as result of standard processing of chromatograms, were used as the base for the quantification purposes.

Gas chromatography/mass spectrometry (GC/MS). The same chromatographic conditions as those mentioned for GC/FID were employed for GC/MS analysis, using HP G 1800C Series II GCD system [Hewlett-Packard, Palo Alto, CA (USA)]. Instead of hydrogen, helium was used as carrier gas. Transfer line was heated at 260°C. Mass spectra were acquired in EI mode (70 eV), in the range of 40-450 Da. Sample solutions were injected by ALS (2 µl, split mode 1:30).

The constituents were identified by comparison of their mass spectra to those from Wiley275 and NIST/NBS libraries, using different search engines (PBM and NIST). In addition, the experimental values for retention indices were determined by the use of calibrated Automated Mass Spectral Deconvolution and Identification System software (AMDIS ver. 2.64.) [6], compared to those from available literature [7], and used as additional tool to approve MS findings.

Results and discussion

The chromatogram obtained by the GC-MS analysis is shown (Figure 1), while the constituents of caraway essential oil are shown in Table 1. From the obtained results it can be concluded that the main constituents of the caraway essential oil are carvone with 68.22% and limonene with 21.80% in content (Table 1).

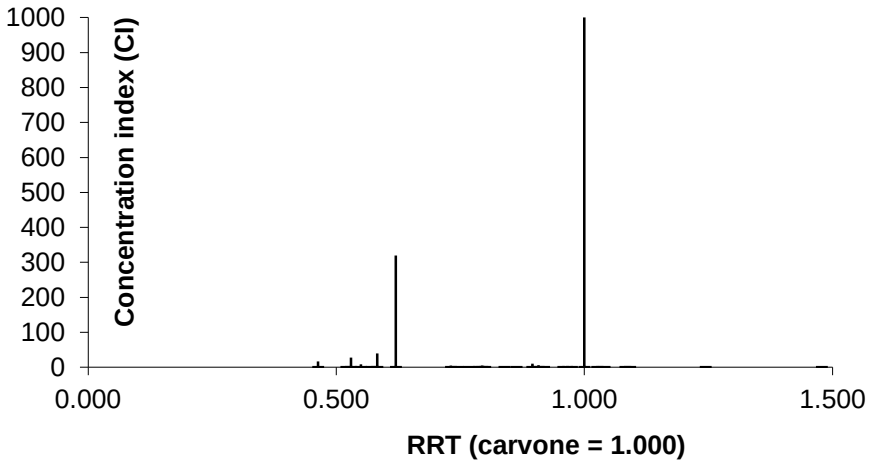

Figure 1. Chromatogram of caraway essential oil

Table 1. Constituents of the caraway essential oil

No	Constituents	KIE	KIL	RT/MS	RT/FID	Area	%, m/m	% ID	RRT	CI
1	□-Pinene	926.6	932	6,57	10,767	841,4	**1,12**	1,12	0,464	16
2	Verbenene	965.5	961	7,73	12,095	74,4	**0,10**	0,10	0,521	1
3	□-Pinene	969.2	974	7,84	12,304	1411,4	**1,88**	1,88	0,530	28
4	Myrcene	988.6	988	8,42	12,765	415,9	**0,56**	0,56	0,550	8
5	□-Phellandrene	1000.7	1002	8,79	13,272	59,6	**0,08**	0,08	0,571	1
6	□^{3}-Carene	1004.8	1008	8,93	13,533	1995,1	**2,66**	2,66	0,583	39
7	Limonene	1025.3	1024	9,63	14,405	16323,3	**21,80**	21,80	0,620	320
8	Linalool	1101.9	1095	12,20	16,981	217,4	**0,29**	0,29	0,731	4
9	trans-Sabinene hydrate	1108.5	1098	12,47	17,313	10,6	**0,01**	0,01	0,745	0
10	trans-p-Mentha-2,8-dien-1-ol	1118.9	1119	12,84	17,844	148,1	**0,20**	0,20	0,768	3
11	cis-Limonene oxide	1128.2	1132	13,16	18,282	164,5	**0,22**	0,22	0,787	3
12	trans-Limonene oxide	1133.4	1137	13,35	18,446	237,0	**0,32**	0,32	0,794	5
13	Camphor	1136,5	1141	13,46	18,588	19,2	**0,03**	0,03	0,800	0
14	Pinocarvone	1157,0	1160	14,17	19,483	70,7	**0,09**	0,09	0,839	1
15	trans-2-Caren-4-ol	1174,3	1176	14,77	20,046	68,7	**0,09**	0,09	0,863	1
16	Myrtenal	1190,6	1195	15,39	20,788	532,0	**0,71**	0,71	0,895	10
17	trans-Dihydro carvone	1199,1	1200	15,63	21,077	292,1	**0,39**	0,39	0,908	6
18	trans-3-Caren-2-ol*	1203,3	n/a	15,77	21,330	112,4	**0,15**	0,15	0,918	2
19	cis-Carveol	1223,3	1226	16,45	22,242	202,8	**0,27**	0,27	0,958	4
20	neoiso-Dihydro carveol	1231,9	1226	16,74	22,640	210,0	**0,28**	0,28	0,975	4
21	Carvone	1247,3	1239	17,26	23,225	51088,7	**68,22**	68,22	1,000	1000
22	Perilla aldehyde	1276,6	1269	18,27	23,835	178,1	**0,24**	0,24	1,026	3
23	n.i.	n/a		n/a	24,165	29,0	**0,04**		1,040	1
24	n.i.	1312,3		19,44	25,142	69,9	**0,09**		1,083	1
25	n.i.	1325,1		19,87	25,361	21,3	**0,03**		1,092	0
26	trans-Caryophyllene	1408,3	1417	22,54	28,904	53,4	**0,07**	0,07	1,244	1
27	Caryophyllene oxide	1573,0	1582	27,47	34,339	36,8	**0,05**	0,05	1,479	1
						74883,9	**100,00**	99,84		

Conclusion

Based on the chromatographic analysis of caraway essential oil obtained in the distillation by water vapor it can be concluded as follows:

- The main constituents of the caraway essential oil are carvone with 68.22% and limonene with 21.80% in content.
- Beside carvone and limonene there were 25 constituents which in total make less than 10% of the studied essential oil.
- Since the main components affect the biological activity of essential oil the conclusion is that the biological effect of caraway essential oil is affected by monocyclic monoterpenes carvone and limonene.

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