

Optimization of HS/GC-MS Method for the Determination of Volatile Compounds from Indigenous Rosemary

Sonia Ancuța Socaci, Maria Tofană, Carmen Socaciu, Cristina Semeniuc

University of Agricultural Sciences and Veterinary Medicine, 3-5 Mănăștur street, 400372 Cluj-Napoca, Romania

Abstract

The volatile compounds from rosemary dried leaves were extracted and then separated and identified by gas-chromatography coupled with mass-spectrometry. Different quantities (0.15g; 0.5g; 1g) of dried rosemary leaves were incubated at various temperatures (30°C; 40°C; 60°C; 75°C; 80°C; 95°C) and periods of time (15'; 30'; 40'). A number of 4 to 28 compounds were separated and identified from rosemary dried leaves, depending on the used method. In all cases, the major compound of rosemary was α -pinene (46.52 – 72.45%), followed by camphene (6.54 - 14.03%), 3-octanone (2.97 – 7.84%), and eucalyptol (2.94 – 8.87%). By increasing the incubation temperature, the incubation time or the quantity of necessary sample may be reduced, the obtained results being similar with those obtained for the same incubation temperature but for a larger incubation time or quantity of sample.

Keywords: Head-space analysis, GC-MS analysis, rosemary, volatile compounds.

1. Introduction

Herbs and spices are invaluable resources, useful in daily life as food additives, flavours, fragrances, pharmaceuticals, colours or directly in medicine [1]. The medicinal properties of plants can be related in part to the presence of volatile compounds. Essential oils are complex mixture of volatile substances generally present in low concentrations. Before such substances can be analyzed, they have to be extracted from matrix. Different methods can be used for this purpose, e.g. hydro-distillation, steam distillation, Soxhlet extraction, solvent extraction, etc. The disadvantages of commonly used sample-preparation techniques are that they require large amounts of organic solvents, or tend to be destructive in nature (i.e. significant artifact formation can occur owing to sample decomposition at high temperature) [2; 3; 4].

Headspace sampling is well suited for fractionation of volatile compounds from complex solid matrix such as plants.

Headspace gas chromatography (HSGC) combines sampling in a headspace of closed container, for example, the space occupied by gases or vapor in a sample vial, with analysis of the sampled gases or vapor by gas chromatography. Recent trends in HSGC applications have shifted to agricultural and food science, pharmaceutical and medical analysis, criminal justice and law, and environmental analysis [2; 6].

The aim of this paper was to optimize the HS/GC-MS method in order to obtain a better extraction of volatile compounds from rosemary leaves, and then separate and identify them by gas-chromatography coupled with mass-spectrometry. This study is one of the steps made by our laboratory in the attempt of developing and optimizing a gas-chromatographic method for the determination of the so called “fingerprint” of different essential oil from indigenous aromatic and medicinal plants.

2. Materials and Method

The analyses were carried out in the Food Quality and Safety Testing Laboratory (FQSTL) from the University of Agricultural Science and Veterinary Medicine from Cluj-Napoca (USAMV).

Plant material. The rosemary samples were collected from the green-house of the Faculty of Agriculture of USAMV. The rosemary leaves were air dried in a cool dark place.

HS analysis. Different quantities (0.15g; 0.5g; 1g) of dried rosemary leaves were incubated at various temperatures (30°C; 40°C; 60°C; 75°C; 80°C; 95°C) and periods of time (15'; 30'; 40'). The HS methods will be codified as follows:

- **HS1:** m=1g; T= 30°C; t= 40 min.
- **HS2 :** m= 1g ; T= 40°C; t= 40 min.
- **HS3 :** m= 1g ; T= 60°C; t= 40 min.
- **HS4 :** m= 1g ; T= 60°C; t= 30 min.
- **HS5 :** m= 1g ; T= 75°C; t= 15 min.
- **HS6 :** m= 0.5g ; T=75°C; t=15 min.
- **HS7 :** m= 0.5g ; T=80°C; t=15 min.
- **HS8:**m= 0.15g ; T=95°C; t=15 min.

GC-MS analysis. The rosemary samples were analyzed by GC-MS. The analyses

were carried out on a Shimadzu GC-MS QP-2010 model gas chromatograph – mass spectrometer equipped with an AOC-5000 autosampler (CombiPAL). An Alltech, USA, AT-5 capillary column of 30m x 0.25mm i.d. and 0.25µm film thickness was used for the analysis. The parameters for the method were: injector temperature 250.0°C; pressure 37.1 kPa; linear velocity 32.4 cm/s; split ratio 1:200. Carrier gas helium; detector: MS, ion source temperature 250.0°C; interface temperature 250.0°C. MS mode: EI. Mass range: 40-400u. Scan speed: 769u/s. The column oven temperature was: 60°C (5 min) to 160°C with 4°C/min to 240°C (1min) with 15°C/min. The identification of separated compounds was made based on the comparison of the obtained mass spectra with the ones from the mass spectra libraries NIST27 and NIST147.

3. Results and Discussion

The number of separated and identified compounds varies between 4 and 28, based on the HS method that was used for the rosemary leaves analysis (table 1).

Table 1. The determined volatile compounds from rosemary leaves using the HS/GC-MS methods

Compound	Retention time (min)	Concentration % (from total peaks area)							
		HS1	HS2	HS3	HS4	HS5	HS6	HS7	HS8
N.I. ^a	7.67	-	-	0.79	0.84	0.58	0.52	0.44	0.32
N.I. ^a	7.84	-	-	-	-	0.12	0.31	0.07	-
α-Pinene	8.10	72.45	67.27	61.82	60.12	62.47	58.62	56.14	46.52
Camphene	8.66	14.03	12.72	11.50	11.69	8.85	8.55	8.01	6.54
trans-Verbenol	8.88	-	-	-	-	0.27	0.26	0.23	0.26
β-Pinene	9.75	-	3.48	3.26	3.60	2.11	2.94	1.66	1.05
1-Octen-3-ol	9.84	-	-	-	-	-	-	-	0.10
3-Octanone	10.15	7.46	2.97	3.68	3.65	4.02	3.67	5.89	7.84
β-Myrcene	10.32	-	3.84	4.38	4.39	5.83	6.94	6.25	5.57
3-Octanol	10.52	-	-	-	-	-	-	0.12	0.26
α-Phellandrene	10.87	-	-	1.61	1.71	2.06	2.53	2.33	2.03
(+)-4-Carene	11.37	-	-	0.72	0.67	0.90	0.92	1.02	0.91
o/m-Cymene	11.67	-	4.10	3.41	3.70	2.85	2.36	4.05	4.81
Limonene	11.86	-	2.68	3.00	3.12	3.72	4.25	4.69	4.88
Eucalyptol	11.98	6.06	2.94	3.42	3.83	3.13	3.54	4.39	8.87
N.I. ^a	12.26	-	-	-	-	0.11	0.15	0.11	0.08
γ-Terpinene	13.12	-	-	0.76	0.85	0.66	1.31	0.94	0.81
Terpinolene	14.34	-	-	-	-	0.23	0.37	0.33	0.42

Table 1. (continuation)

Compound	Retention Time (min)	Concentration % (from total peaks area)							
		HS1	HS2	HS3	HS4	HS5	HS6	HS7	HS8
β -Linalool	14.80	-	-	-	-	-	0.10	0.11	0.31
N.I. ^a	14.95	-	-	-	-	-	-	-	0.03
N.I. ^a	15.82	-	-	-	-	-	-	-	0.12
Camphor	16.62	-	-	1.66	1.82	1.54	1.87	2.53	6.18
Borneol	17.47	-	-	-	-	0.55	0.45	0.57	1.24
N.I. ^a	17.91	-	-	-	-	-	-	0.05	0.15
α -Terpineol	18.43	-	-	-	-	-	-	-	0.15
Verbenone, (I)	19.18	-	-	-	-	-	-	0.08	0.29
Ylangene	25.22	-	-	-	-	-	-	-	0.03
Caryophyllene	26.91	-	-	-	-	-	-	-	0.10
α -Caryophyllene	28.05	-	-	-	-	-	-	-	0.10

^a – not identified

As it can be observed from table 1, the number of separated and identified compounds increases with the increase of incubation temperature. Thus, when the rosemary leaves were incubated at 30°C for 40', only four compounds were separated, while at the incubation temperature of 95°C for 15', 28 compounds were separated. In all cases, the major compound of rosemary was α -pinene (46.52 – 72.45%), followed by camphene (6.54 – 14.03%), 3-octanone (2.97 – 7.84%), and eucalyptol (2.94 – 8.87%).

The compounds that were separated from rosemary leaves incubated at 30°C were: α -pinene, camphene, 3-octanone and eucalyptol, thus meaning that these four compounds are the most volatile from rosemary, giving the characteristic smell of the plant.

The concentration of α -pinene, camphene, β -pinene decreases with the increase of temperature (from 30°C to 95°C), due to the fact that with the increase of temperature other volatile compounds are extracted from rosemary leaves and pass in the gaseous phase (headspace).

The higher concentration of eucalyptol was obtained for the incubation temperature of 95°C, even if the sample weight was only 0.15g, while in the other cases was 1g or 0.5g. At lower incubation temperatures (40°C - 60°C) the peak of eucalyptol is well separated from the peak of limonene, while at higher incubation temperatures (75°C - 95°C) the two peaks aren't so well

separated (figure 1 and 2). The ratio between the limonene concentration and eucalyptol concentration is 1:1 in the case of incubation temperatures between 40°C and 80°C, and 1:2 in the case of incubation temperature of 95°C.

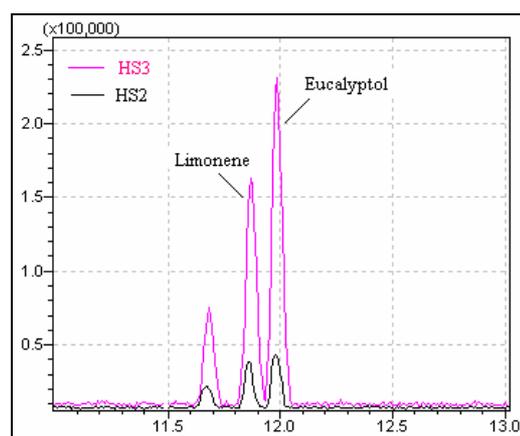


Figure 1. The peaks corresponding to eucalyptol and limonene, obtained with method HS2 and HS3 (low incubation temperatures)

The camphor content increases with the increase of incubation temperature. Thus, at high incubation temperatures (80°C - 95°C) the camphor concentration (2.53% - 6.18%) is from two to six times higher than that obtained in the case of use of low incubation temperatures methods (1.54% - 1.87%).

In the case of high incubation temperatures methods, especially HS8, seven new compounds were separated comparing with the HS7 method: 1-octen-3-ol, α -terpineol, ylangene, caryophyllene, α -caryophyllene and two not identified compounds.

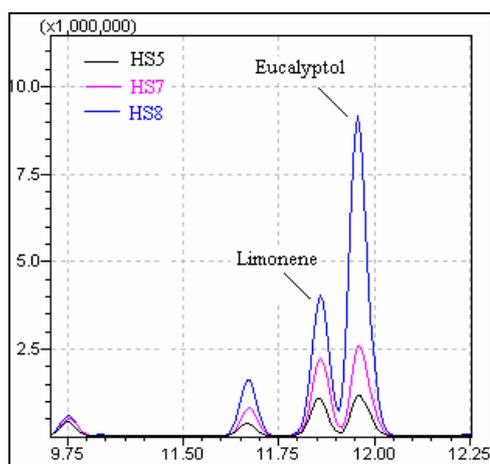


Figure 2. The peaks corresponding to eucalyptol and limonene, obtained with method HS5, HS7 and HS8 (high incubation temperatures)

Analysing the data from table 1 and the obtained chromatograms (figure 3) it can be said that by increasing the incubation temperature, the incubation time may be reduced (HS3 and HS4).

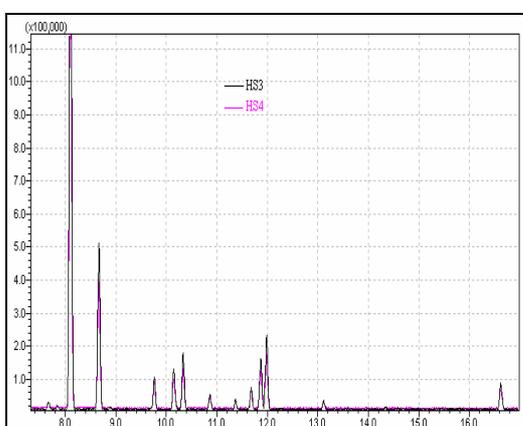


Figure 3. The HS/GC-MS chromatogram rosemary dried leaves obtained by using HS3 method (40° of incubation) and HS4 method (30° of incubation)

If we take in consideration the weight of the sample, it can be noticed that by increasing the incubation temperature, the quantity of necessary sample may be reduced, the obtained results being similar (method HS5 and HS6 – figure 4).

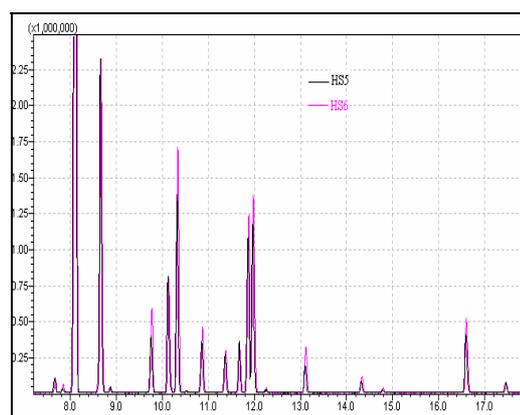


Figure 4. The HS/GC-MS chromatogram rosemary dried leaves obtained by using HS5 method (1g of sample) and HS6 method (0.5g of sample)

The main compound of rosemary essential oil analyzed by GC-MS in our previous studies [7] was α -pinene followed by camphor, eucalyptol, camphene, limonene, 3-octanone and β -myrcene. From qualitative point of view, all the above mentioned compounds were found in the dried rosemary leaves using HS/GC-MS methods. From quantitative point of view, there are some differences regarding the concentration of the separated compounds that will be analyzed and used for the optimization of the HS/GC-MS method.

4. Conclusions

A number of 4 to 28 compounds were separated and identified from rosemary dried leaves, depending on the used method. The number of separated and identified compounds increases with the increase of incubation temperature, the higher number of separated compounds being obtained with method HS8 (95°C – incubation temperature).

In all cases, the major compound of rosemary was α -pinene (46.52 – 72.45%), followed by camphene (6.54 - 14.03%), 3-octanone (2.97 – 7.84%), and eucalyptol (2.94 – 8.87%).

At lower incubation temperatures (40°C - 60°C) the peak of eucalyptol is well separated from the peak of limonene, while at higher incubation temperatures (75°C -

95°C) the two peaks aren't so well separated.

By increasing the incubation temperature, the incubation time may be reduced (HS3 and HS4). Also, by increasing the incubation temperature, the quantity of necessary sample may be reduced, the obtained results being similiary (method HS5 and HS6).

In the future, the results obtained by HS/GC-MS methods for the rosemary leaves composition in volatile compounds will be compared with those obtained for the rosemary essential oil.

Acknowledgements

This research financially supported by the TD-108 grant: "Optimizarea sistemelor GC-MS pentru determinarea amprentei uleiurilor volatile din plante aromatice si medicinale" financed by UEFISCSU.

References

1. Lucchesi, Marie E., F. Chemat, Jacqueline Smadja, Solvent-free microwave extraction of essential oil from aromatic herbs: comparison with conventional hydro-distillation, *J. Chromatogr. A*, 2004, 1043, 323-327.
2. Huie, Carmen W., 2002, A review of modern sample-preparation techniques for extraction and analysis of medicinal plants, *Anal. Bioanal. Chem.*, 373, 23-30
3. Setkova, Lucie, Sanja Risticovic, J. Pawliszyn, 2007a, Rapid headspace solid-phase microextraction – gas chromatographic-time – of – flight mass spectrometric method for qualitative profiling of ice wine volatile fraction. I. Method development and optimization, *Journal of Chromatography*, 1147, 213-223
4. Setkova, Lucie, Sanja Risticovic, J. Pawliszyn, 2007a, Rapid headspace solid-phase microextraction – gas chromatographic-time – of – flight mass spectrometric method for qualitative profiling of ice wine volatile fraction. II. Clasification of Canadian and Czech ice wines using statistical evaluation of the data, *Journal of Chromatography*, 1147, 224-240
5. Lo Presti, Maria, S. Ragusa, Alessandra Trozzi, Paola Dugo, F. Visinoni, Alessia Fazio, G. Dugo, L. Mondello, 2005, A comparison between different techniques for the isolation of rosemary essential oil, *J. Sep. Sci.*, 28, 273-280
6. Zhu, J.Y., X-S. Chai, 2005, Some recent developments in headspace gas chromatography, *Current Analytical Chemistry*, 1, 79-83
7. Sonia A. Socaci, Maria Tofana, Carmen Socaciu, 2008, GC-MS analysis of rosemary essential oil, *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca*, 65 (2), 405-409