

Volatile Constituents of Leaves and Flowers of *Thymus mastichina* by Headspace Solid-Phase Microextraction

V. ALMEIDA^{1,4}, V. GONÇALVES¹, L. GALEGO¹, G. MIGUEL², M. COSTA³

⁽¹⁾ Escola Superior de Tecnologia da Universidade do Algarve, Campus da Penha, 8005 - 139 FARO (Portugal)

⁽²⁾ Faculdade de Engenharia de Recursos Naturais da Universidade do Algarve, Campus de Gambelas, 8005 -139 FARO (Portugal)

⁽³⁾ Direcção Regional de Agricultura do Algarve, Apartado 282, Braciais – Patação, 8001 – 904 FARO (Portugal)

⁽⁴⁾ Instituto de Tecnologia Química e Biológica, Quinta do Marquês – Apartado 127, 2780-505 OEIRAS (Portugal)

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Abstract

In the present work, the volatile compounds of leaves and flowers of *Thymus mastichina* collected in two different places of Portugal were studied using head space solid-phase micro-extraction with gas chromatography-mass spectrometry (HS-SPME-GC-MS) procedure. The analysis fell upon dried leaves and flowers directly or by the essential oils obtained by hydrodistillation.

The results showed that linalool (26 -30 %) was the most important compound in leaves and flowers of plants from Sesimbra. In Algarve plants, 1,8-cineol or eucalyptol was the most significant volatile compound of which concentrations ranged from (23 – 33 %). In general these results are in accordance with that already studied by hydrodistillation and GC-MS.

INTRODUCTION

Steam distillation and solvent extraction methods combined with GC or GC-MS are used as the routine methods for the analysis of the volatile essential oils of aromatic plants. However, these conventional methods for the analysis of the essential oils have some disadvantages. Steam distillation requires a relative high amount of sample and is a time consuming procedure. Solvent extraction has the disadvantage that it also extracts non-volatile resinous components along with the essential oils, which adversely affect the GC column and the recovery of the volatile components is greatly influenced by the extraction conditions (Song *et al.*, 2003). Solid phase microextraction (SPME) technology introduced in 1990 by Arthur and Pawliszyn (Arthur & Pawliszyn, 1990) is a simple and solvent-free technique. Gradually SPME with GC-MS has been applied to the determination of the volatile chemical components of several aromatic plants.

In traditional Portuguese cooking and medicine several aromatic plants have been used. Lamb meat, kid or rabbit are normally aromatized with thyme. Thyme is also used for preparing tea. There are several species of *Thymus* in Portugal which essential oil composition varies deeply. The goal of this study was to compare volatile profile of

Thymus mastichina grown in two different regions of Portugal using different analytic techniques of extraction. One of the methods was hydrodistillation, as reported in European Pharmacopoeia, and the other one was headspace solid phase micro-extraction (HS-SPME). In both methods, the chemical analysis was performed by gas-chromatography coupled to mass spectrometry (GC-MS).

MATERIALS AND METHODS

Isolation of essential oils

The oils were isolated from dried materials by hydrodistillation, for 4 hours, using a Clevenger-type apparatus (Anonymous, 1996).

Solid phase micro extraction procedure

0,5 g of dry plant were introduced into a 20 ml vial. Two different coated fibers were used a 65 µm PDMS-DVB (polydimethylsiloxane – divinylbenzene) and a 50/30 µm DVB-Carboxen-PDMS.

The SPME fiber was exposed 20 min in the head-space at laboratory temperature (20 ± 2 °C), after the fiber was withdrawn into the needle and transferred to the injector of the GC-MS, where the analytes were thermally desorbed from the fiber during 5 or 8 min depending if it was a PDMS-DVB or a DVB-Carboxen-PDMS respectively.

GC-MS analysis

A Shimadzu 17-A chromatograph equipped with Shimadzu QP-500 mass spectrometer was used. The separation was achieved using a J&W Scientific DB-1701P column of 30 m x 0,25 mm i. d. and 0,25 µm of film thickness. GC oven temperature was programmed from 40 °C (5 min), to 230 °C at a rate of 5 °C/min and then 5 min at 230 °C. The carrier gas was helium with a column-head pressure of $1,4 \times 10^5$ Pa.

Mass spectra were recorded in the electron impact (EI) mode at 70 eV, scanning the m/z 30 to 300. Interface temperature was 250 °C. Data acquisition and data processing were carried using Class5K programme.

Peaks in TIC (total ion current) or MIC (Multi Ion Chromatogram) profiles for both analyses were characterized or tentatively identified from their mass spectral data using National Institute of Standards and Technology (NIST12 or NIST62) and Wiley 229 mass spectrometry libraries. Identification was confirmed using standard compounds when available.

GC analysis

Gas chromatographic analysis was carried out using a Hewlett Packard 5890 Série II equipped with a FID detector. Helium was used as the carrier gas. The components were separated on 30 m x 0,25 mm i.d., 0,25 µm film thickness DB-1701P column from J & W Scientific. The injector temperature was set at 250 °C and all injections were made in split mode (split, 30:1). The column was initially maintained at 50 °C for 5 minutes; subsequently the temperature was increased to 210 °C at a rate of 5 °C/min and finally held for 5 minutes. FID Detector temperature was set at 270 °C. Data acquisition and data processing were carried using *Chromulan* programme.

RESULTS AND DISCUSSION

Figure 1 compares total ion chromatogram of a HS-SPME-GC-MS analysis of dried samples of *Thymus mastichina* (A) and total ion chromatogram of a HS-SPME-GC-MS analysis of *Thymus mastichina* oil obtained by hydrodistillation (B). Both are from Algarve region.

The results show that there is a good agreement between the components obtained by hydrodistillation and those obtained by HS-SPME. In both methods, eucalyptol (Salgueiro, 1994) was the main component of the samples. This was already expected since several studies have shown that *Th. mastichina* plants from Algarve are particularly rich in this component (Salgueiro, 1994; Miguel *et al.*, 2003). The great difference was observed for isobornyl acetate and caryophyllene that were almost present as traces in the essential oil while in dried plant they were present in relative higher amounts. The isolation of essential oils by hydrodistillation has some disadvantages with the possibility of occurring artefacts or formation of crystals on the condenser surface (Cavaleiro, 2001). This is the reason by which some devices associate a steam distillation with a simultaneous continuous extraction (Chaintreau, 2001). In spite of this preliminary assay demonstrates a good relation between the methods, it was also clear that the amounts of minor components may differ. In this case and in biological terms the utilization of plants or their essential oils can contribute to different activities, since synergism and/or antagonism effects between major and minor components may occur and be responsible for diverse activities. However, other parameters must be evaluated in the HS-SPME in order to optimize the conditions of work. For *Angelica* species, some authors (Pawliszyn, 1999) found that Carbowax-divinylbenzene (CW-DVB) fiber was more suitable for the absorption of volatile constituents than PDMS fiber. The same authors also showed that the optimum extraction conditions with this fiber were 60 °C during 30 min. This is in accordance of that already reported by some authors that refer the temperature has a significant effect on the kinetic of the process (Pawliszyn, 1999).

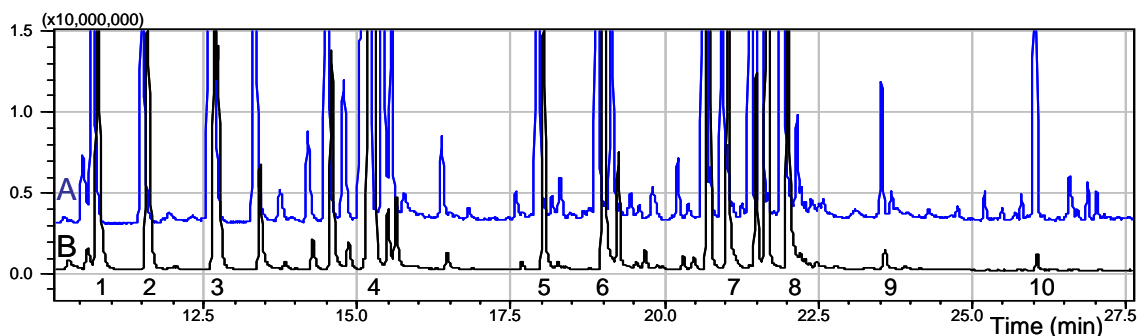


Fig 1: Total ion chromatograms by HS-SPME with PDMS-DVB fiber: dried sample (above - A), oil obtained by hydrodistillation (down - B); Identification of some important compounds: 1 – α -Pinene; 2- Camphene; 3- β -Pinene; 4- 1,8-cineol or Eucalyptol; 5 – Sabinene hydrate; 6 - Linalool; 7 – Terpinen-4-ol; 8 – Borneol; 9 – Isobornyl acetate; 10- Caryophyllene.

The profiles of total ion chromatogram of both HS-SPME-GC-MS analysis of dried samples and essential oils were practically similar in the samples collected in Sesimbra (Estremadura) as reported above for the samples from Algarve. However in those samples the main component was linalool (Fig. 2A). Such results were already

reported by Salgueiro (Salgueiro, 1994) in plants collected in the same region, some years ago.

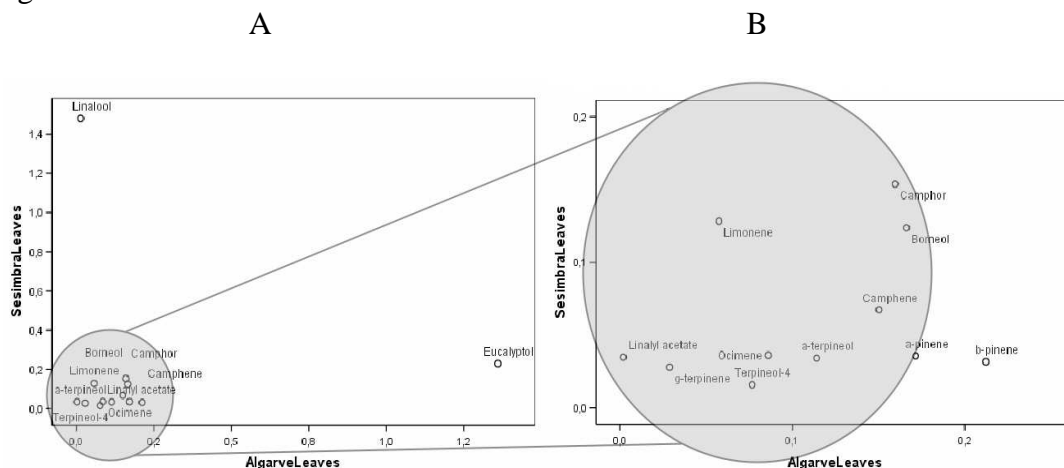


Fig 2 Comparison of *Thymus mastichina* leaves from Sesimbra with *Thymus mastichina* leaves from Algarve

In Fig 2B it is also possible to verify that the bicyclic hydrocarbon monoterpenes like α -pinene or β -pinene are present in higher concentrations in the essential oils of the plants from Algarve than in those from Sesimbra. Such results may reveal a higher capacity of these plants to cyclize the monoterpenes than the plants from Sesimbra, where acyclic monoterpenes predominate.

Acknowledgements

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