

Headspace solid-phase microextraction gas chromatography and gas chromatography – mass spectrometry analysis of the volatile compounds during packing of: *Calamintha baetica*, *Thymus mastichina* and *Origanum vulgare*

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ABSTRACT

Aromatic plants have been used since ancient times, in food flavouring, pharmaceutical or cosmetics due to the presence of essential oils. The present work reports volatile compounds of dried *Calamintha baetica*, *Thymus mastichina* and *Origanum vulgare* collected in an experimental field of “Direcção Regional de Agricultura do Algarve” (DRAALG). The volatile compounds had been studied by headspace solid-phase microextraction with gas chromatography and gas chromatography-mass spectrometry (HS-SPME-GC-MS) procedures. Analyses were done after drying and after 6 month in different kinds of packing (glass, low density polyethylene, polypropylene, cotton woven and craft paper).

The results show, that in the same conditions, the most volatile compounds (bicyclic monoterpenes hydrocarbons and 1-8 cineol) increase their relative percentages, on the other hand, less volatile compounds (sesquiterpenes like β -caryophyllene, α -bisabolene, germacrene-d or phenols like carvacrol or thymol and oxygenated like linalool) decrease after 6 months of storage in every kind of packing. Due to the great number of variables that influence the characteristics of the different aromatic plants multivariate analyses were considered by using the NTSYS software, and it is possible to observe that glass and polypropylene present more similarity after 6 months of storage in all kinds of packing. If this preliminary studies to continue significative differences will arise with the increase of time storage to one or two years.

Introduction

Calamintha baetica, *Thymus mastichina* and *Origanum vulgare* are aromatic plants grown up spontaneously in mountainous areas of Algarve and are used traditionally in food preparations. *Calamintha baetica*, or *Calamintha sylvatica* (www.pfaf.org) is typically named olive-herb because it is mainly used to aromatize a typical local olive preservation, *Thymus mastichina* was employed to make tea for stomach disease and *Origanum vulgare* was added into tomato salad, with boiled snails, in olive preservation, and in other specific gastronomic dishes.

Traditionally people cut the plants, dry them at room temperature and store them in different packing but they don't know which is the best packing for these plants.

Conventional methods such as steam distillation or solvent extraction 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 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), and normally cause pollution problems to the environment and to the technicians. Solid phase microextraction (SPME) technology introduced in 1990 by Arthur and Pawliszyn (Arthur & Pawliszyn, 1990: 2145) is a simple and solvent-free technique based in sorption (adsorption and/or absorption, depending on the fibre coating) which is used for extraction and concentration analyses either by submersion in liquid phase or by exposure to a gaseous phase (Arthur *et al.* 1992: 1960). This technique has become popular in the analysis of volatile and semivolatile chemicals as its superiority over conventional methods has been recognized (Ai, 1997: 1230)

Material and methods

Plant material

Plants grew up in the experimental field of "Direcção Regional de Agricultura do Algarve" (DRAALG), all of them in three different blocks with 25 plants each, in order to get a representative sample. All these plants are endogenous of Algarve. After flowering, plants were cut and dried in a solar drier in the same conditions. Some samples were immediately analysed after drying and others were stored in 5 different kinds of packing: glass, low density polyethylene, polypropylene, cotton woven and craft paper.

Solid phase micro extraction procedure

Exactly 0,5 g of dry plant were introduced into a 20 ml *vial*. A 65 μm PDMS-DVB (polydimethylsiloxane – divinylbenzene) coated fibre was used. The SPME fibre was exposed 20 min in the head-space at laboratory temperature (20 ± 2 °C), after the fibre was withdrawn into the needle and transferred to the injector of the GC and/or GC-MS, where the analytes were thermally desorbed from the fibre during 5 min.

GC-MS analysis

A Shimadzu 17-A chromatograph equipped with Shimadzu QP-5000 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 held 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 software.

Peaks in TIC (total ion current) or MIC (Multi Ion Chromatogram) profiles 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 were 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 using *Chromulan* software.

Results and Discussion

Figure 1 shows the volatile profile compounds of the 3 plants studied after 6 month of storage, obtained by HS-SPME-GC.

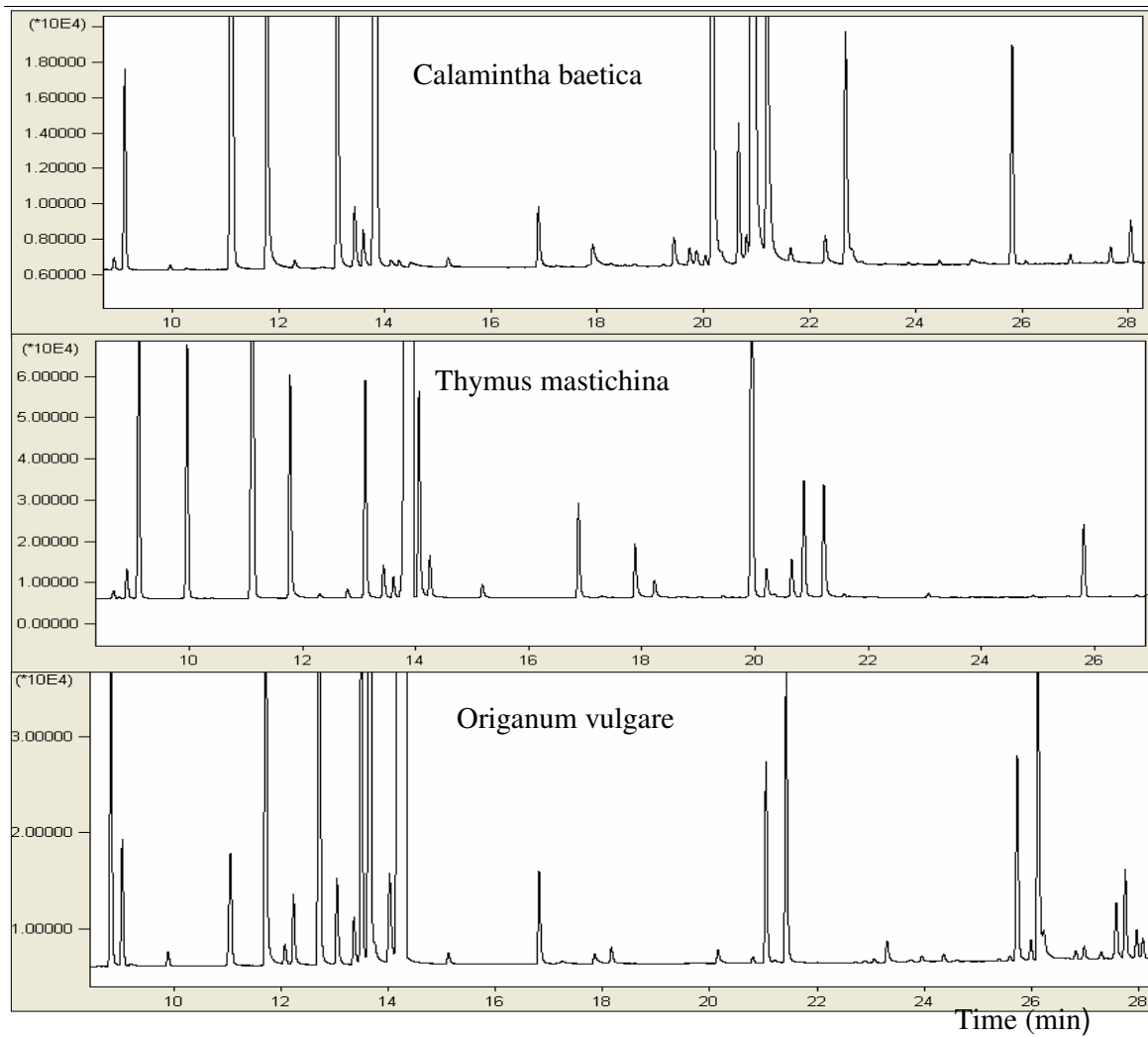


Fig 1: Volatile compounds of the three plants we are studying, after 6 months of storage, analysed by HS-SPME-GC.

Some volatile compounds are present in all the plants but their concentration differs, and other compounds are typical of the specimen. All of them have a group of more volatile compounds that eluted before 15 minutes of analysis, essentially monoterpenes and a second group after 25 minutes of analyse, essentially sesquiterpenes.

Figure 2 presents a volatile profile of a sample of *Calamintha baetica*, or *Calamintha sylvatica* made GC after 6 months of packing in craft paper with the identification of the main compounds.

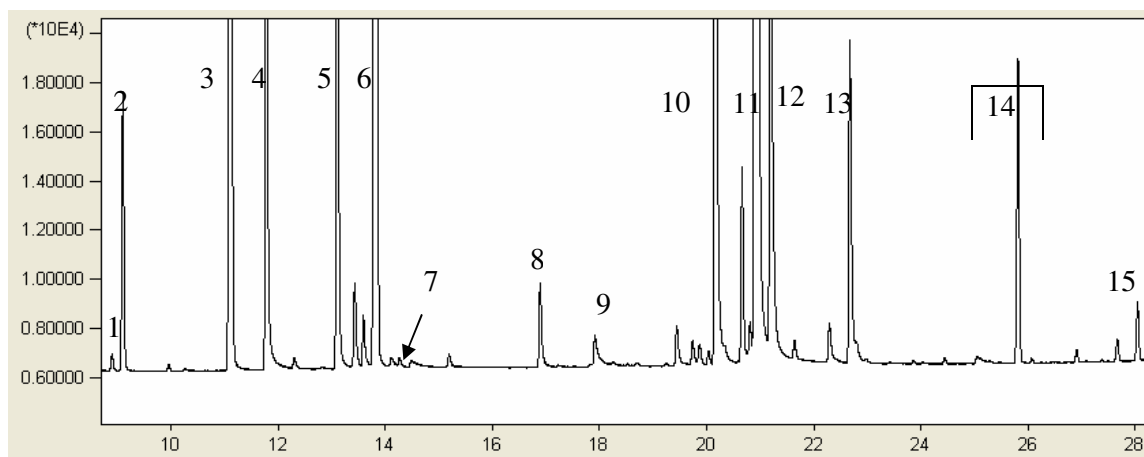


Fig 2: *Calamintha baetica*. Identification: 1–Thujene; 2 – α -pinene; 3– Sabinene + β pinene; 4– Myrcene; 5– Limonene; 6– 1-8 Cineol or Eucalyptol; 7– γ – terpinene; 8– trans-sabinene-hydrate; 9– α -terpinolene; 10– Isopulegol; 11– Isopulegone; 12– α – terpineol; 13– Pulegone; 14– Trans-Caryophyllene; 15– Bicyclo-germancrene.

Figure 3 compares the first group of compounds (blue) immediately after drying and after 6 months stored in glass (black). It's important to stress γ -terpinene because it decreased greatly after 6 months of storage in every kind of packing. On the other hand α -pinene, β -pinene + sabinene and myrcene increase their concentration for the same period of storage.

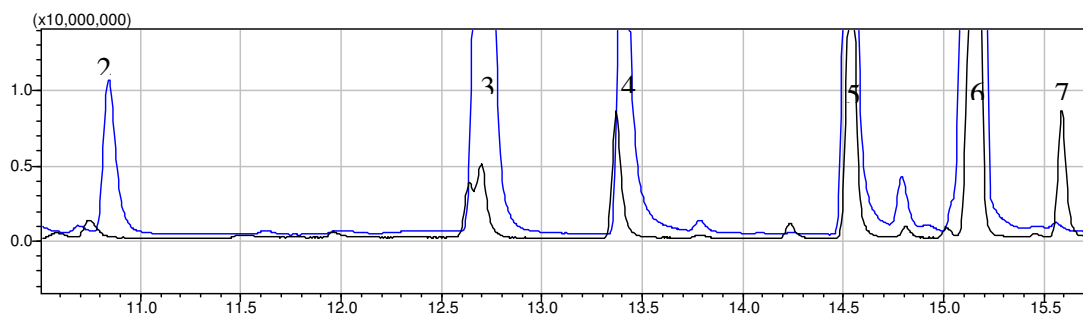


Fig 3: Comparison of *Calamintha baetica* immediately after drying (blue) and after 6 month of storage in glass packing (black).

All the results were analysed by NTSYS software, using PCA and Cluster analysis. The first and the second principal component account for more than 80% of the total variance. Euclidean distances were selected as a measurement of similarity, and the unweighted pair-group method with arithmetic average (UPGMA) was used for cluster definition. Figure 4 shows an image obtained by analysing 30 variables from the six samples used as the average of three replicates. One sample corresponds to the plant analysed immediately after drying and the other five correspond to the five different kinds of packing used with *Thymus mastichina*.

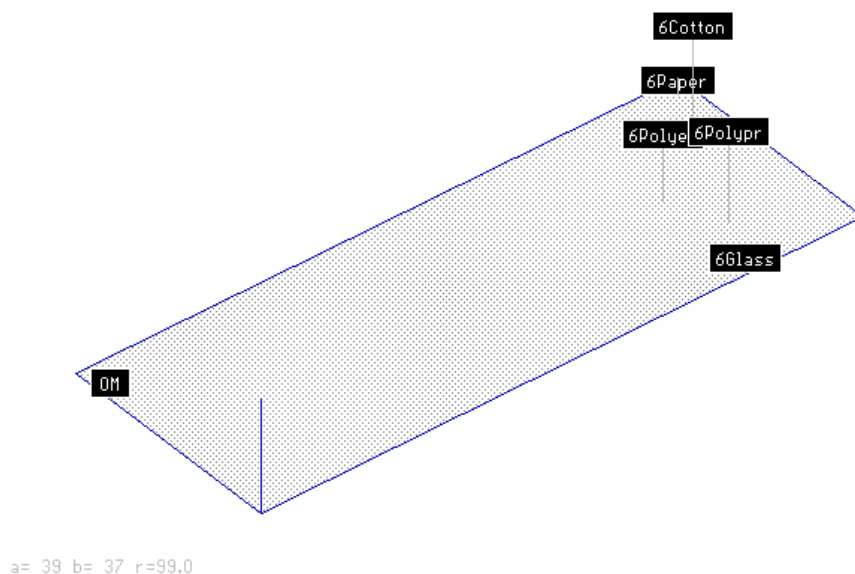


Fig 4: *Thymus mastichina* plotting of the first three principal components of characteristics immediately after drying (OM) and after 6 months of storage in different kind of packing (6 glass, 6 polypropilene, 6 polyetilene, 6 cotton, 6 paper)

Comparison of *Thymus mastichina* immediately after drying (upper – black) and after 6 month of storage in glass (red) or cotton (blue) packing, can be seen in figure 5.

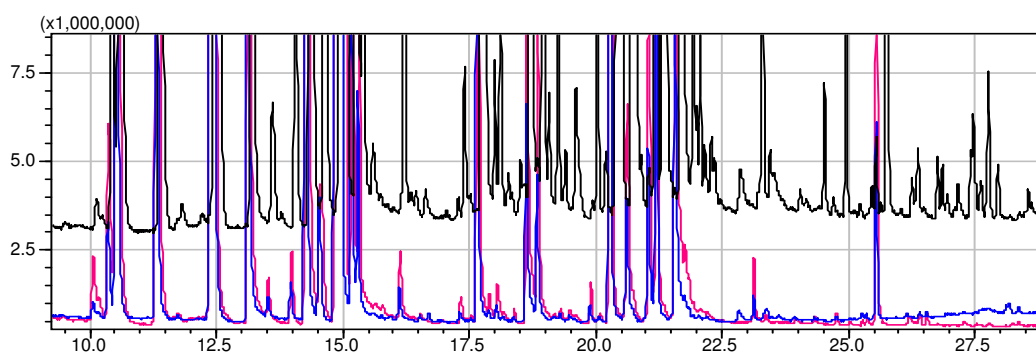


Fig 5: Comparison of *Thymus mastichina* immediately after drying (upper - black) and after 6 month of storage in glass (red) or cotton (Blue) packing.

With the analytical conditions used, a greater intensity of compounds can be seen located at the end of the graphic immediately after drying when compared with the ones after 6 months of storage in all kinds of packing.

In all the plants studied differences are observed in the total volatile compounds after 6 months of storage in every kind of packing used.

Figure 6 and figure 7 were obtained using NTSYS software for the *Origanum vulgare* after six months of storage in different kinds of packing.

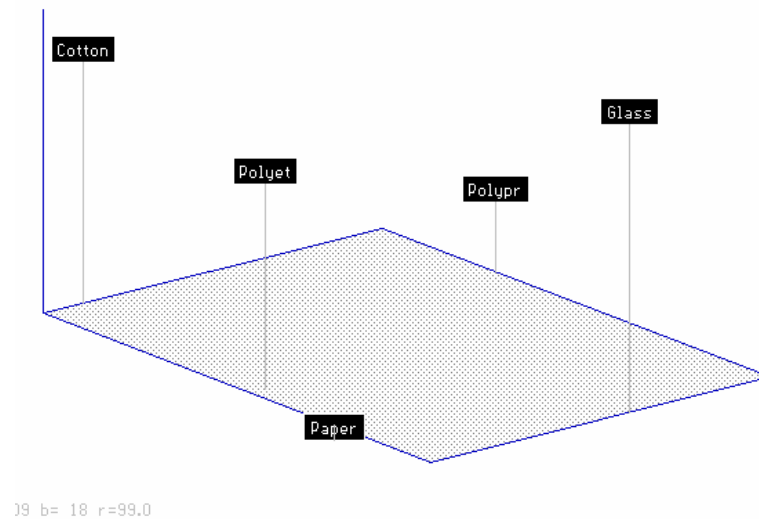


Fig 6: *Origanum vulgare* plotting of the first three principal components of characteristics after 6 months of storage in different kinds of packing (glass, polypropylene, 6 polyethylene, cotton, paper)

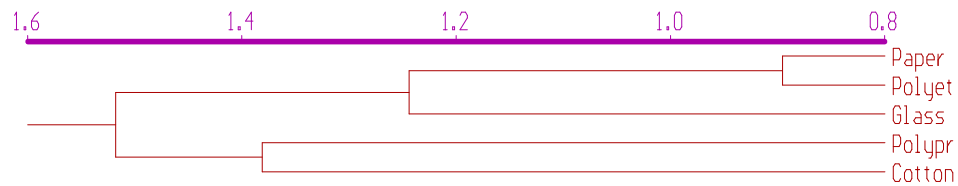


Fig 7: *Origanum vulgare* dendrogram obtained by cluster analysis of characteristics after 6 months of storage in different kinds of packing (glass, polypropylene, polyethylene, cotton, paper) based in euclidean distances and unweighted pair-group method with arithmetic average (UPGMA).

Six months reveals not to be enough time to observe significant differences in the all kinds of packing used although it is possible to see a tendency that separates paper and polyethylene in a group and polypropylene and cotton in other group.

These preliminary studies already show differences, with the increase of time of storage of one or two years greater differences are expected to arise.

Acknowledgements

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