Determination of volatile organic compounds of *Tagetes filifolia* Lag. (Asteraceae) from Córdoba (Argentina) using HS-SPME analysis

[Determinación de compuestos orgánicos volátiles de *Tagetes filifolia* Lag. (Asteraceae) proveniente de Córdoba (Argentina) utilizando análisis por HS-SPME]

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Abstract

A headspace solid-phase microextraction (HS-SPME) method followed by gas chromatography-mass spectrometry (GC-MS) and gas chromatography-flame ionization detection (GC-FID) are described for the analysis of volatile compounds in *Tagetes filifolia* Lag. The composition of the total aerial parts of the plant (whole plant) and the inflorescences, leaves and stems were studied separately using HS-SPME. As a result, 54 compounds were determined, of which 47 were identified. The major components observed in this analysis were trans-anethole and estragole. The HS-SPME method used for the analysis of volatile compounds of *T. filifolia* is simple, fast, effective, free from the use of solvents, and permits by an analysis of small amounts of samples to achieve better results in terms of the determination of the composition than those reported in the literature for the analysis of essential oil.

Keywords: HS-SPME; *Tagetes filifolia* Lag; volatile organic compounds

Resumen

Se realizó el análisis de los componentes volátiles de *Tagetes filifolia* Lag. utilizando el método de microextracción en fase sólida del espacio de cabeza con análisis posterior por cromatografía de gases acoplada a espectrometría de masas y por cromatografía de gases con detección por ionización de llama. Se estudió la composición de la planta entera así como también la de las inflorescencias, hojas y tallos por separado empleando el método de HS-SPME. Como resultado, se determinaron 54 compuestos de los cuales 47 fueron identificados. Los componentes mayoritarios observados en este análisis fueron: trans-anetol y estragol. El método de HS-SPME utilizado para el análisis de los compuestos volátiles de *T. filifolia* es simple, rápido, efectivo, libre de la utilización de solventes, y permitió mediante el análisis de pequeñas cantidades de muestra alcanzar mejores resultados en cuanto a la determinación de la composición, que los reportados en literatura para el análisis del aceite esencial.

Palabras Clave: HS-SPME; *Tagetes filifolia* Lag.; compuestos orgánicos volátiles.

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LISTA DE ABREVIACIONES


INTRODUCTION

The genus Tagetes (Asteraceae) is currently assigned to about 50 species throughout the American continent. In Argentina, this genus is represented by 12 species, of which 5 are endemic. In the Province of Cordoba, three species are found growing wild in the mountainous area, with one of these being Tagetes filifolia Lag., which is commonly known as “Anisillo”, “Anis del campo” or “Anis de las Sierras”. This is an annual herb, with an aniseed odor and low height (30 cm), which is widely distributed in the mountains of Cordoba and San Luis and in others Argentine provinces (Ariza Espinar, 1967; Pettenati and Ariza Espinar, 1997).

T. filifolia has been used to reduce stomach and menstrual pains, as fodder for livestock and as an insecticide (Serrato Cruz et al., 2006). In this latter case, the use of essential oil extracted from T. filifolia has a wide potential application against nematodes and insect biotransmitters of viruses (Cubillo et al., 1999; Serrato Cruz, 2003; Serrato Cruz et al., 2005). The low cost of production of essential oil (Serrato Cruz, 2003) coupled with good yields and its organic origin, make it an important ecological and economical alternative to synthetic insecticides.

Some studies using GC-MS/FID analyses have described the composition of essential oils obtained by hydrodistillation of samples of T. filifolia and certain differences have been observed: 1) the number of compounds varies from 33 (Vila et al., 2000) to 57 (Feo et al., 1998), 2) cis-anethole (Feo et al. 1998) and trans-anethole (Zygadlo et al., 1993, Vila et al., 2000) have been found, and 3) the amounts of estragole and anethole in the total composition of the essential oil have been reported as 61.2% and 33.1% (Vila et al., 2000), 21% and 79% (Serrato Cruz et al., 2005), 13.7% and 68.3% (Feo et al., 1998) or 29.5% and 68.1% (Maestri et al., 1991), respectively. This variability in the chemical composition of the essential oil of T. filifolia may be due to environmental differences between the places of origin of the material analyzed, since this species is found growing wild throughout America. Nevertheless, in all this cases estragole and anethole constitute most of the essential oil of T. filifolia (Maestri et al., 1991; Zygadlo et al., 1993; Feo et al., 1998; Vila et al., 2000). Different biological effects such as a repellent, insecticide, fungicide, nematicide and antibacterial have been found for estragole and anethole, and for essential oil obtained from others plant families such as Lamiaceae and Apiaceae than have estragole and anethole as active compounds (Tuzun and Yegen, 2000).

Analysis by gas chromatography of the essential oils obtained by hydrodistillation is the most common methodology employed to characterize the volatile organic compounds present in aromatic plants (Saroglou et al., 2006; Magwa et al., 2006). However, the hydrodistillation methodology of isolation of essential oils is a time-consuming and laborious process and requires large amounts of samples. Moreover, when investigators extract essential oils from a plant matrix for analysis, little attention is paid to the possibility that the extraction methods may yield different essential oil profiles, or even worse, a sample degradation, despite it being well known that chemical reactions can occur during the distillation process (Babu and Kaul, 2007). For this reason, the final composition of the product may not be representative of the original material, and the observed variations in oil composition may strongly depend on the type of distillation method used (Babu et al., 2002; Babu et al., 2004; Babu et al., 2005). Thus, it is important that researchers explore the various advantages and disadvantages of a given extraction or instrumental technique before carrying out the analysis.

Solid-phase microextraction (SPME), developed by Pawliszyn and co-workers (Arthur et al., 1990) is proving to be increasingly useful in organic analytical chemistry due to it being a rapid and simple procedure of extraction with a great capacity of concentration without the need for any organic solvent (Cai et al., 2006; Vas and Vékey, 2004). This technique has been used for the direct extraction of volatile compounds in matrices such as vegetables, fruits, juices, soft drinks and alcoholic beverages (Kataoka et al, 2000). Consequently, this has the advantage of minimizing the sample handling, and reduces the loss of volatile compounds that normally can occur during the hydrodistillation process for obtaining essential oils. Moreover, it is simple and fast modern tool, which has been used to characterize the...
volatile fraction of aromatic and medicinal plants with
good results (Marriott et al., 2001; Smith, 2003) and
offers a valid alternative to hydrodistillation to carried
out gas chromatographic analysis of volatile
constituents from different sources.

To date, SPME analysis of volatile
constituents on whole plants of T. filifolia and their
aerial parts has not been reported in the literature to
our knowledge. For this reason, the present study
describes qualitative and semi-quantitative
determinations of volatile compounds of T. filifolia
(whole plant and aerial parts separately) using
analytical techniques the HS-SPME/GC–MS and HS-
SPME/GC-FID. Additionally, a comparison was made
between the results obtained by HS-SPME and those
reported in the literature for essential oil analyses.

MATERIALS and METHODS
Plant Samples
Specimens of T. filifolia Lag., in the process of
flowering were collected in February 2010 in the
Sierras Grandes de Córdoba, Argentina. A whole plant
has been deposited in the Herbarium Marcelino
Sayago, Faculty of Agricultural Sciences, Catholic
University of Córdoba (Deposit Number
UCCOR399b).

To perform HS-SPME/GC-FID and HS-
SPME/GC-MS analyses, samples (100.0 ± 0.1 mg) of
the fresh aerial parts of several plants previously
chopped up with a clean cutter were placed in glass
vials of 20 cm³, which were then closed with Viton
septa and aluminium seals provided by Supelco
(Sigma-Aldrich, Argentina).

The vials containing the samples were
immersed in a thermostatic water bath at 40 °C
(PolyScience 8005, accuracy 0.2 °C). After 10 min, the
SPME device was inserted into the sealed vial by
manually penetrating the septum, and the fiber was
exposed to the sample headspace for 30 min. After
extraction, the needle on the SPME manual holder was
set to its maximum length in the GC injector and the
fiber was directly exposed to the hot injector at 250 °C
for 5 min in splitless mode.

Selection of extraction fiber and HS-SPME
optimization
The selection of the fiber used and the conditions of
equilibrium time of the HS, extraction temperature and
extraction time (exposure time of the fiber used for the
HS) for each analysis were previously established by
the measurement and characterization of the volatile
compounds present in samples of Tagetes minuta L.

(Vázquez et al., 2011). Using a manual holder
(Supelco), the fiber was conditioned in the GC injector
at 225 °C for 8 hours before use.

In study of fresh aerial parts of T. filifolia were
finally adopted an extraction temperature of 40 °C and
equilibrium time of 10 min for HS, and a extraction
time of 30 min for the SPME.

Gas Chromatography (GC)
Analyses were performed using a gas chromatograph
Shimadzu GC14B, equipped with a flame ionization
detector, a manual injection port operating in a
splitless mode and an HP-5 capillary column (30 m x
0.25 mm ID x 0.25 μm film). Working conditions were:
injector: 225 °C; detector temperature: 230 °C;
gas carrier: N₂; 99.99% and pressure of column head: 5
psi. Temperature programming of column from 40 °C
(5 min) to 200 °C (5 min); heating rate: 5 °C/min. The
percentages of the compounds were determined by
normalizing the peak area of the chromatogram with
respect to the total area. All analyses were performed
by triplicate and the variation coefficient of relative
areas was less than 5%.

Gas Chromatography-Mass Spectrometry
The identification of volatile components was carried
out using a HP 5890 Series II gas chromatograph
equipped with a manual injection port operating in a
splitless mode and coupled to a HP 5970 Mass
Detector. The column used was a HP-5 capillary
column (30 m x 0.25 mm ID x 0.25 μm film).
Working conditions were: injector: 225 °C; interface:
230 °C; gas carrier: He; 99.99%; pressure of column
head, 5 psi. Temperature programming of column
from 40 °C (5 min) to 200 °C (5 min); heating rate: 5
°C/min. The mass spectrometer was operated at 70 eV
and spectra were recorded in the range of m/z 25-550
amu in the acquisition mode "scan-full". The data
processing system used was HP-MS ChemStation
including database Wiley 275 and NIST. The volatile
components were identified by comparing their mass
spectra with library data (Match ≥ 90%) and through
the determination of the respective Kovat retention
indices (KI), (alkanes standards provided by Sigma-
Aldrich). These retention indices were compared with
those reported in the NIST (2011) database and
Pherobase (2011).
RESULTS AND DISCUSSION

**HS-SPME of combined aerial parts of the whole plant**

As can be seen in Table 1, the existence of 46 different components in the volatile fraction of *T. filifolia* were established, 43 of which were successfully identified (93.48%). Thus, positive identification was achieved in 99.72% of the total area observed in the chromatogram.

Table 1 summarizes the main components provided by the HS-SPME analysis of the aerial parts of the whole plant, with estragole (22.3%) and trans-anethole (57.06%) being found at the greatest percentages. In addition, the following compounds were also observed, but at smaller amounts: α-farnesene (2.36%), anisic aldehyde (1.98%), α-bisabolene (1.33%), α-isocoumarin (1.22%), isoledene (1.19%) and valencene (1.03%). All the rest of the components were present at amounts ranging from 0.05% (δ-maaliene) to 0.99% (δ-cadinene).

### Table 1
Composition of volatile organic compounds of *Tagetes filifolia* Lag. using HS-SPME/GC-MS/FID analysis.

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<th>Rt (min)</th>
<th>Compounds*</th>
<th>KI&lt;sub&gt;e&lt;/sub&gt;</th>
<th>KI&lt;sub&gt;r&lt;/sub&gt;</th>
<th>I:</th>
<th>L:</th>
<th>S:</th>
<th>WP:</th>
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HS-SPME of inflorescences
As can be seen in Table 1, the existence of 42 different components in the volatile fraction of the inflorescence of *T. filifolia* were established, 38 of which were successfully identified (90.48%). Thus, positive identification was achieved in 99.48% of the total area observed in the chromatogram.

Estragole (16.34%) and trans-anethole (51.16%) were found to be the major components of the results obtained on inflorescences (Table 1). Additionally, the presence of appreciable amounts of α-farnesene (4.88%), germacrene D (3.13%), α-bisabolene (2.53%), δ-cadinene (2.0%), β-thujone (1.1%) and δ- elemene (2.30%) were determined. At smaller proportions, bicyclogermacrene (1.84%), α-amorphene (1.56%), anisic aldehyde (1.50%), γ-cadinene (1.48%), α-muurelene (1.15%) and epi-bicyclosesquiphellandrene (1.03%) were also found. The rest of the observed components were present at amounts ranging from 0.11% (β-longipinene and isocaryophyllene) to 0.96% (α-isocomicene).

HS-SPME of leaves
As can be seen in Table 1, the existence of 41 different components in the volatile fraction of the leaves of *T. filifolia* were established, 39 of which were successfully identified (95.12%). Thus, positive identification was achieved in 99.6% of the total area observed in the chromatogram.

From measurements made on leaves of *T. filifolia* (Table 1), the major components were estragole (27.15%) and trans-anethole (61.8%). Lower proportions were observed of α-farnesene (2.25%) and bicyclogermacrene (1.05%), with the rest of the components being present at amounts ranging from 0.03% (α-ylangene) to 0.64% (δ-cadinene).

HS-SPME of stems
As can be seen in Table 1, the existence of 32 different components in the volatile fraction of the leaves of *T. filifolia* were established, 26 of which were successfully identified (81.25%). Thus, positive identification was achieved in 99.46% of the total area observed in the chromatogram.

In stems of *T. filifolia* (Table 1), estragole (19.74%) and trans-anethole (38.05%) were observed to be the main components. Moreover, isoledene (7.84%), α-bisabolene (7.59%), α-isocomicene (6.75%), valencene (4.60%), β-elemene (4.01%) and β-caryophyllene (2.63%) were observed at appreciable amounts. The rest of the components were present at amounts ranging from 0.05% (trans-α-bergamotene) to 0.72% (bicyclogermacrene).

Differences between the composition of inflorescences, stems and leaves
The data summarized in Table 1 show some interesting differences among the results of the HS-
SPME analyses of inflorescences, stems and leaves of *T. filifolia*:

1) There was a significant difference in the number of compounds produced by each part of the plant. Both the inflorescences and the leaves were responsible for the greatest number of volatile organic compounds (42 and 41 respectively), while the stems contributed a smaller number (only 32 compounds).

2) The main components estragole and *trans*-anethole were found at greater proportions in leaves than in inflorescences or stems. However, the stems were the main source of α-cubebene, isosedene, α-isocamphone, β-elemene, β-caryophyllene, and α-bisabolene.

3) 1-undecene, duraldehyde, β-maaliene, and α-humulene were minor compounds and were only present in stems. Moreover, whereas *cis*-3-hexenol and γ-muurolene were exclusive components of the leaves, α-muurolene was only observed in the inflorescences.

4) The contribution of methyl eugenol, α-copaene, iso-longipholen, aristolene, *allo*-aromadendrene, α-elemene, calarene, *epi*-bicycloesquiphellandrene, α-amorphene, germacrene D, α-zingiberene, α-farnesene, cadina-1,4-diene, α-calacorene and δ-gurjrene to the composition of volatile organics observed in the whole plant was exclusively due to inflorescences and leaves. Similarly, the contribution of *trans*-α-bergamotene and α-bisabolene also came exclusively from inflorescences and stems.

5) Additionally, valencene was found exclusively in leaves and stems.

**HS-SPME analysis vs. essential oil analysis**

In data reported in the literature (Aburrá *et al.*, 1990; Maestri *et al.*, 1993; Zygadlo *et al.*, 1993), the components present in the essential oil were eleven, of which five were identified. However, using the HS-SPME analysis reported here, 46 components were measured on the aerial parts of the whole plant, of which 43 were identified. Comparing the results obtained for essential oil analysis reported in the literature and the HS-SPME data obtained here, revealed that there was high similitude in the identified compounds, being *trans*-anethole (∼57%) and estragole (∼22%) the majority compounds. However, the HS-SPME analysis demonstrated a better characterization of the volatile organic compounds present in *T. filifolia* than the essential oil analyses, because it was possible to identify a greater number of minority components. Perhaps, these could not be observed by essential oil analyses as they might have been lost during the hydrodistillation process.

**CONCLUSIONS**

A simple, rapid and solvent-free technique to determine the volatile components in *T. filifolia* plants using the HS-SPME-GC–MS/FID methods was established. Using a smaller quantity of samples, a shorter extraction time and a much simpler procedure, with HS-SPME method can achieve better results than those obtained by essential oil analysis. Moreover, HS-SPME method allows the composition of volatile organic compounds from the separate aerial parts to be rapidly determined.

The method can therefore be used in further studies aimed at characterizing different populations of *T. filifolia* by HS-SPME analyses.

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