

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]

Ana M. VÁZQUEZ¹, Mario L. AIMAR², Gabriela I. DEMMEL¹, M. Eugenia CABALEN¹, Silvia G. CRIADO¹, Juan J. CANTERO³, Manuel I. VELASCO⁴ & Laura I. ROSSI⁴

¹Facultad de Ciencias Químicas, Universidad Católica de Córdoba, Córdoba, Argentina.

²Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Córdoba, Argentina.

³Departamento de Biología Agrícola/Facultad de Agronomía y Veterinaria, Universidad Nacional de Río Cuarto, Río Cuarto, Córdoba, Argentina

⁴Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina.

Contactos / Contacts: Ana M. VÁZQUEZ - E-mail address: ana.vazquez.s@gmail.com

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.

Recibido | Received: April 19, 2012.

Aceptado en versión corregida | Accepted in revised form: May 9, 2012.

Publicado en línea | Published online: March 30, 2013

Declaración de intereses | Declaration of interests: AM Vazquez gratefully acknowledges financial support from the Catholic University of Córdoba; LI Rossi and MI Velasco gratefully acknowledge financial support from CONICET, FONCYT and the Secretaría de Ciencia y Tecnología de la Universidad Nacional de Córdoba (SECyT-UNC).

Este artículo puede ser citado como / This article must be cited as: AM Vazquez, ML Aimar, GI Demmel, M Cabalen, SG Criado, JJ Cantero, MI Velasco, LI Rossi. 2013. Determination of volatile organic compounds of *Tagetes filifolia* Lag. (Asteraceae) from Córdoba (Argentina) using HS-SPME analysis. *Bol Latinoam Caribe Plant Med Aromat* 12(2): 143 – 149.

LISTA DE ABREVIACIONES

T. filifolia – *Tagetes filifolia* Lag.; VOCs – Volatile Organic Compounds; EO – essential oil; HS-SPME – Solid Fase Micro Extraction of Headspace; GC – Gas Chromatography; MS – Mass Spectrometry; FID – Flame Ionization Detector; Rt – Retention time; PDMS – polydimethylsiloxano; CAR – carboxene; DVB – divinylbencene; KI_e – experimental Kovat's Index; KI_r – reference Kovat's Index; L. – leaves; S. – stems; I. – Inflorescences; W.P. – Whole Plant.

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 (Zygaldo *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 these cases estragole and anethole constitute most of the essential oil of *T. filifolia* (Maestri *et al.*, 1991; Zygaldo *et al.*, 1993; Feo *et al.*, 1998; Vila *et al.*, 2000). Different biological effects such as a repellent, insecticide, fungicide, nematocidal and antibacterial have been found for estragole and anethole, and for essential oil obtained from other 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 cm^3 , 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 $40 \text{ }^\circ\text{C}$ (PolyScience 8005, accuracy $0.2 \text{ }^\circ\text{C}$). 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 $250 \text{ }^\circ\text{C}$ 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 $225 \text{ }^\circ\text{C}$ for 8 hours before use.

In study of fresh aerial parts of *T. filifolia* were finally adopted an extraction temperature of $40 \text{ }^\circ\text{C}$ 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 ($30 \text{ m} \times 0.25 \text{ mm ID} \times 0.25 \text{ } \mu\text{m}$ film). Working conditions were: injector: $225 \text{ }^\circ\text{C}$; detector temperature: $230 \text{ }^\circ\text{C}$; gas carrier: N_2 99.99% and pressure of column head: 5 psi. Temperature programming of column from $40 \text{ }^\circ\text{C}$ (5 min) to $200 \text{ }^\circ\text{C}$ (5 min); heating rate: $5 \text{ }^\circ\text{C}/\text{min}$. 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 ($30 \text{ m} \times 0.25 \text{ mm ID} \times 0.25 \text{ } \mu\text{m}$ film). Working conditions were: injector: $225 \text{ }^\circ\text{C}$; interface: $230 \text{ }^\circ\text{C}$; gas carrier: He 99.99%; pressure of column head, 5 psi. Temperature programming of column from $40 \text{ }^\circ\text{C}$ (5 min) to $200 \text{ }^\circ\text{C}$ (5 min); heating rate: $5 \text{ }^\circ\text{C}/\text{min}$. 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 $\geq 90\%$) 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 -isocomene (1.22%), isodene (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.

Peak	Rt (min)	Compounds ^a	KI _e	KI _r	% ^b			
					I:	L:	S:	WP:
1	9.298	<i>cis</i> -3-hexenol	839	839	-	0.27	-	0.20
2	17.426	1-undecene	1095	1095	-	-	0.41	0.11
3	21.247	Estragole	1213	1208	16.34	27.15	19.74	22.30
4	22.818	anisic aldehyde	1265	1270	1.50	1.61	1.21	1.98
5	24.155	<i>trans</i> -anethol	1309	1301	51.16	61.80	38.05	57.06
6	24.800	Unknown	1332	-	-	0.15	0.06	-
7	24.848	Eugenol	1334	1337	0.24	0.28	0.18	0.37
8	25.114	Duraldehyde	1343	1340	-	-	0.09	-
9	25.087	δ -elemene	1342	1347	0.32	0.16	0.08	0.28
10	25.315	methyl eugenol	1350	1348	0.71	0.39	-	0.55
11	25.637	α -cubebene	1362	1358	0.67	0.12	1.91	0.43
12	25.620	Unknown	1361	-	-	-	0.13	-
13	26.087	β -maaliene	1378	1381	-	-	0.12	0.05
14	26.263	α -ylangene	1384	1382	0.24	0.03	0.43	0.10
15	26.388	α -copaene	1388	1390	0.32	0.06	-	0.17
16	26.555	Isodene	1394	1392	0.73	0.07	7.84	1.19
17	26.650	β -longipinene	1398	1403	0.11	0.03	-	-
18	26.740	α -isocomene	1401	1405	0.96	0.15	6.75	1.22
21	26.908	<i>iso</i> -longipholen	1407	1406	0.32	0.06	-	0.16
22	27.073	<i>iso</i> -caryophyllene	1413	1414	0.11	0.15	0.46	0.23
23	27.297	β -elemene	1422	1420	0.35	0.06	4.01	0.63
24	27.416	Unknown	1426	-	0.07	-	-	-
25	27.572	β -caryophyllene	1432	1430	0.82	0.17	2.63	0.60
26	27.823	<i>trans</i> - α -bergamotene	1441	1440	0.58	-	0.05	0.27
27	27.939	Clovene	1446	1444	0.32	0.15	0.37	0.09
28	28.024	Aristolene	1449	1450	0.17	0.10	-	0.12
29	28.132	Aromadendrene	1453	1452	0.65	0.40	0.13	0.50
30	28.221	<i>allo</i> -aromadendrene	1456	1459	0.20	0.12	-	0.16
31	28.290	α -elemene	1459	1460	0.40	0.06	-	0.16
32	28.421	<i>trans</i> - β -farnesene	1464	1464	0.55	0.07	0.37	0.19
33	28.484	α -humulene	1466	1465	-	-	0.62	0.22
34	28.504	Calarene	1467	1467	0.36	0.07	-	0.17
35	28.759	<i>epi</i> -bicyclosquiphellandrene	1477	1471	1.03	0.26	-	0.27
36	29.117	α -amorphene	1490	1485	1.56	0.06	-	0.08
37	29.084	γ -muurolene	1489	1489	-	0.27	-	0.61
38	29.165	Valencene	1492	1493	-	0.16	4.60	1.03

39	29.278	germacrene D	1496	1493	3.13	0.46	-	0.69
40	29.452	α -zingiberene	1503	1498	0.30	0.20	-	0.24
41	29.568	α -muurelene	1507	1506	1.15	-	-	0.54
42	29.668	Bicyclogermacrene	1511	1511	1.84	1.05	0.72	0.97
43	29.887	α -farnesene	1520	1519	4.88	2.25	-	2.36
44	29.950	α -bisabolene	1523	1523	2.53	-	7.59	1.33
45	30.124	γ -cadinene	1530	1526	1.48	0.37	0.31	0.58
46	30.333	δ -cadinene	1538	1533	2.30	0.64	0.55	0.99
47	30.556	cadina-1,4-diene	1547	1548	0.30	0.07	-	0.11
48	30.679	α -cadinene	1552	1556	0.54	0.14	0.26	0.21
49	30.829	α -calacorene	1558	1560	0.21	0.09	-	0.12
50	31.324	δ -gurjunene	1577	1571	0.12	0.04	-	0.09
51	31.483	Unknown	1584	-	-	-	0.09	-
52	31.993	Unknown	1604	-	0.23	0.11	0.11	0.15
53	32.582	Unknown	1629	-	0.19	0.14	0.08	0.10
54	33.619	Unknown	1673	-	0.03	-	0.06	0.03
% Total Area					100.00	100.00	100.00	100.00
% Total area identified					99.48	99.60	99.46	99.72
% Compounds identified					90.48	95.12	81.25	93.48

^aBy HS-SPME/GC-MS analysis and determination of Kovat's Index. ^bBy HS-SPME/GC-FID analysis, where I = inflorescence; L = leaves; S = stem and WP = whole plant.

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 *iso*-caryophyllene) to 0.96% (α -isocomene).

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 *estragele* (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), *estragele* (19.74%) and *trans*-anethole (38.05%) were observed to be the main components. Moreover, *isodene* (7.84%), α -bisabolene (7.59%), α -isocomene (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, isodene, α -isocomene, 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*-bicyclosquiphellandrene, α -amorphene, germacrene D, α -zingiberene, α -farnesene, cadina-1,4-diene, α -calacorene and δ -gurjunene 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 ($\approx 57\%$) and estragole ($\approx 22\%$) 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.

ACKNOWLEDGEMENTS

A. M Vazquez gratefully acknowledges financial support from the Catholic University of Cordoba. L. I. Rossi and M. I. Velasco gratefully acknowledge financial support from CONICET, FONCyT and the Secretaria de Ciencia y Tecnología de la Universidad Nacional de Córdoba (SECyT-UNC). We thank Dr Damian Maestri for providing some bibliographical references and Dr Paul Hobson, native speaker, for revision of the manuscript.

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