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# Determination of Volatile Organic Compounds of *Tagetes argentina* Cabrera (Asteraceae) using HS-SPME analysis

[Determinación de Compuestos Orgánicos Volátiles de *Tagetes argentina* Cabrera (Asteraceae) 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) is described for the analysis of volatile compounds in *Tagetes argentina* Cabrera. The composition of the totality of the aerial parts of the plant (whole plant) and the inflorescences, leaves and stems was studied separately using HS-SPME. As a result, 53 compounds were determined, of which 39 were identified. The major components were: *cis*-tagetenone, *trans*-tagetenone, *trans*-tagetone and *cis*-tagetone.Using a much smaller amount of samples, a shorter extraction time and a very simple procedure, the HS-SPME method can achieve similar or better results than those obtained by EO analysis. In conclusion, the HS-SPME method is simple, rapid, effective and free of solvent, and can be used for the analysis of volatile compounds in samples of different populations of *T. argentina*.

**Keywords:** HS-SPME; *Tagetes argentina* Cabrera; volatile organic compounds.

**Resumen**
Se realizó el análisis de los componentes volátiles de *T. argentina* Cabrera utilizando el método de micro-extracción 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 estudio la composición de la totalidad de las partes aéreas de la planta (planta entera) como así también de las inflorescencias, hojas y tallos por separado empleando el método de HS-SPME. Como resultado, se determinaron 53 compuestos de los cuales 39 fueron identificados. Los componentes mayoritarios fueron: *cis*-tagetenona, *trans*-tagetenona, *trans*-tagetona and *cis*-tagetona. Utilizando una muy pequeña cantidad de muestra, un corto periodo de tiempo y un procedimiento muy simple se lograron similares o mejores resultados a aquellos reportados mediante el análisis del aceite esencial. En conclusión, el método de HS-SPME es simple, rápido, efectivo, libre de la utilización de solventes y puede ser fácilmente implementado para el análisis de componentes volátiles provenientes de muestras de diferentes poblaciones de *T. argentina*.

**Palabras Clave**: HS-SPME; *Tagetes argentina* Cabrera; compuestos orgánicos volátiles.

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**List of Abbreviations***: T. argentina – Tagetes argentina* Cabrera.; 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.

## INTRODUCTION

In Argentina, the genus *Tagetes* (Asteraceae) is represented by 12 species, of which 5 are endemic (Ariza Espinar, 1967; Barboza *et al.*, 2006). *Tagetes argentina* Cabrera is one of three species found in the province of Córdoba, growing wild in the mountainous area at an altitude between 1000 at 2500 meters above sea level (Katinas and Ariza Espinar, 1999). Commonly known as "Chil chil del campo" or "Chinchigua", this plant is native and is found mainly in the mountains of Cordoba and San Luis. However, it has also been reported in areas northwest of Mendoza (Ariza Espinar, 1967). It frequently occurs in wet places and as a weed among crops (Gutiérrez and Katinas, 2003) and it has been used as a diuretic and digestive aid in folk medicine (Barboza *et al.*, 2006).

The chemical composition of *T. argentina* essential oils has been previously studied (Zygadlo *et al.*, 1993a, 1993b) and the main constituents identified were *cis*-tagetenone (43.62-45.59%) and *trans*-tagetenone (37.29-40.38%). Lesser amounts of *cis*-β-ocimene (1.46-1.49%), *trans*-β-ocimene (2.00-7.26%), dihydrotagetone (3.82-6.57%), *cis*-tagetone (1.29-1.77%) and *trans*-tagetone (1.30-1.87%) were also found in this oil.

Although hydrodistillation is the most common extraction technique employed to obtain essential oils from aromatic plants (Saroglou *et al*., 2006, Magwa *et al*., 2006, Becerra *et al*., 2010; Moreno *et al*., 2010; Urzua *et al*., 2010; Buitrago *et al*., 2011), it is a laborious and time-consuming process that 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 (Babu and Kaul, 2007). For this reason, the final composition of the product may not be representative of the original material (Babu *et al*., 2002; 2004; 2005). Thus, it is important that researchers explore the various advantages and disadvantages of a given extraction or instrumental technique before carrying out an analysis.

Solid-phase microextraction (SPME) was introduced by Pawliszyn and co-workers (Arthur, 1990), with this technique proving increasingly useful in organic analytical chemistry due to it being a rapid procedure of extraction without the requirement of any organic solvent (Cai *et al*., 2006; Vas and Vékey, 2004), and having the advantage of minimizing the sample handling and consequently decreasing the loss of volatile compounds. Furthermore, it is a simple and fast modern tool which has been previously used to characterize the volatile fraction of aromatic and medicinal plants (Marriott *et al*., 2001; Smith, 2003). This technique, therefore offers a valid alternative to hydrodistillation for gas chromatographic analysis of volatile constituents from different sources.

To our knowledge, there are no reports in the literature about HS-SPME analysis of the volatile constituents of whole plants of *T. argentina* or their aerial parts separately. For this reason, the present study describes both qualitative and semi-quantitative determinations of volatile compounds of *T. argentina* using the analytical techniques HS-SPME/GC–MS and HS-SPME/GC-FID. Additionally, a comparison is made between the results obtained by HS-SPME and those reported in the literature for essential oil analyses.

## MATERIALS AND METHODS

### Plant Samples

Several specimens of *T. argentina* Cabrera in the process of flowering-fruiting were collected between March and April 2010 in the Sierras Grandes de Córdoba, Argentina. A whole plant has been deposited in the Herbarium Marcelino Sayago (Register Number UCCOR 336d), Faculty of Agricultural Sciences, Catholic University of Córdoba.

To perform the analysis of HS-SPME/GC-FID and HS-SPME/GC-MS, samples (100.0 ± 0.1 mg) of fresh aerial parts previously chopped up were placed in glass vials of 20 $cm^3$. These were then sealed with Viton septa and aluminum seals provided by Supelco (Sigma-Aldrich, Argentina).

### Selection of extraction fiber and HS-SPME optimization

As a part of a major program on the study of the flora in the Province of Córdoba, a project was commenced with the aim of characterize the composition of volatile fraction of the different species of *Tagetes* that grown in our Province.

In this sense, our team developed an analytical methodology using HS-SPME/GC-MS and HS-SPME/GC-FID to determine the composition of the

volatile organic compounds present in *Tagetes minuta* L. (Vázquez *et al.*, 2011). That study determined that the best conditions of work were achieved using a fiber of DVB-CAR-PDMS of 50/30 μm (Sigma-Aldrich of Argentina). Therefore, due to the great similarity in the composition of the main volatile organic components present in *T. minuta* and *T. argentina* (Zygadlo *et al.*, 1993b), it was decided in the present study to use the same working conditions we previously used with *T. minuta* to carry out this study to determine the volatile organic compounds present in *T. argentina*.

Using a manual holder (Supelco), the fiber was first conditioned in a GC injector at 225º C for 8 hours. Then, the vials containing the samples were immersed 10 mm in a thermostatic water bath at 40° C (PolyScience 8005, accuracy ± 0.2º C). After 10 min, an SPME device was inserted into the sealed vial by manually penetrating the septum, and the fiber was exposed to the plant material 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 250º C for 5 min in splitless mode

***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 a ZB-5 capillary column (30 m x 0.25 mm ID x 0.25 μm film). The working conditions were: injector: 225º C; initial temperature: 40º C (5 min); final temperature: 200º C (5 min); heating rate: 5º C / min; detector: 230º C; carrier gas: $N_2$ 99.99% and head pressure: 5 psi, and the percentage composition was established by normalizing the peak area of the chromatogram with respect to the total area. All determinations were performed in triplicate and the variation coefficient was less than 5%.

**Gas Chromatography-Mass Spectrometry**

The identification of the volatile components was performed using a gas chromatograph HP 5890 Series II equipped with a manual injection port operating in a splitless mode. This was coupled to an HP 5970 Mass Detector and the column used was an HP-5 capillary column (30 m x 0.25 mm ID x 0.25 μm film). The working conditions were: injector: 225º C; initial temperature: 40º C (5 min); final temperature: 200º C (5 min); heating rate: 5º C/min; interface: 230º C, gas carrier: He 99.99%; head pressure: 5 psi. The mass spectrometer was operated at 70 eV and the spectra were recorded in the range of m/z 25-550 amu in the acquisition mode "scan-full." The data processing system used was the HP-MS ChemStation, including database Wiley 275 and NIST. The volatile components were identified by comparing their mass spectra with library data and by the determination of the respective Kovat´s retention indices (KI), (alkane standards provided by Sigma-Aldrich). The Retention indices were compared with those reported in the databases (NIST, 2010; Pherobase, 2010).

**RESULTS AND DISCUSSION**

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

As can be seen in Table 1, the existence of 41 different components in the volatile fraction of *T. argentine* were established, 32 of which were successfully identified (78%). Thus, positive identification was achieved in 99.1% 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 *cis*-tagetenone (17.9 %) and *trans*-tagetenone (28.8%) being found at the greatest proportions. In addition, there were also appreciable amounts of: *trans*-tagetone (11.8%), *cis*-tagetone (9.4%), dihydrotagetone (6.4%), bycyclogermacrene (3.0%), *trans*-β-ocimene (2.6%) and germacrene D (2.5%), β-caryofilene (2.3%). The presence of p-cymen-8-ol (1.7%), piperitone (1.3%), verbenone (1.1%), aromadendrene (1.0%) and calarene (1.0%) was also determined, but to a lesser degree. The rest of the observed components were present at amounts ranging from 0.9% (limonene) to 0.1% (for example terpineol, among others).

***HS-SPME of inflorescences***

As can be seen in Table 1, the existence of 39 different components in the volatile fraction of the inflorescence of *T. argentine* were established, 37 of which were successfully identified (82.1%). Thus, positive identification was achieved in 99.5% of the total area observed in the chromatogram.

*Cis*-tagetenone (29.6%) and *trans*-tagetenone (21.1%) were found to be the major components by the results obtained on inflorescences (Table 1). Additionally, the presence of appreciable amounts of *cis*-tagetone (10.1%), *trans*-tagetone (8.8%), dihydrotagetone (5.2%), *trans*-β-ocimene (3.0%), bicyclogermacrene (2.9%), germacrene D (2.4%), β-caryophyllene (2.1%), p-cymen-8-ol (2.0%), β-thujone

(1.1%) and δ- elemene (1.1%) were determined. At smaller proportions, piperitone (1.6%), verbeneone (1.3%), aromadendrene (1.1%) and anisole (1.1%) were also found. The rest of the observed components were present at amounts ranging from 0.9% (calarene) to 0.1% (for example spathulenol, among others).

***HS-SPME of leaves***

As can be seen in Table 1, the existence of 38 different components in the volatile fraction of leaves of *T. argentine* were established, 33 of which were successfully identified (86.9%). Thus, positive identification was achieved in 99.7% of the total area observed in the chromatogram.

From measurements made on leaves of *T. argentina* (Table 1), the major components were *cis*-tagetenone (33.3%) and *trans*-tagetenone (14.4%). Lower proportions were observed of 4-terpineol (10.0%), *trans*-tagetone (7.7%), *cis*-tagetone (7.2%), carvone (6.0%), dihydrotagetone (2.8%), p-cymen-8-ol (1.5%), aromadendrene (1.5%), germacrene D (1.2%), bicyclogermacrene (1.2%) and verbenone (1.1%). The rest of the components were present at amounts ranging from 0.9% (for example α-amorphene) to 0.1% (for example methyleugenol, among others).

***HS-SPME of stems***

As can be seen in Table 1, the existence of 25 different components in the volatile fraction of the leaves of *T. argentina* were established, 21 of which were successfully identified (84.0). Thus, positive identification was achieved in 99.0% of the total area observed in the chromatogram.

In stems of *T. argentina* (Table 1), dihydrotagetone (29.6%) and β-caryophyllene (13.5%) were observed to be the main components. At smaller proportion: *cis*-tagetone (8.2%), *trans*-tagetone (7.9%), calarene (5.6%), bicyclogermacrene (5.4%), germacrene D (4.5%), *cis*-tagetenone (4.1%), *trans*-β-ocimene (3.3%), *allo*-cimene (3.1%), *trans*-tagetenone (2.8%) and δ-cadinene (1.8%) were found. The rest of the observed components were present at amounts ranging from 0.9% (γ-cadinene) to 0.3% (*cis*-β-ocimene).

***Differences between the composition of inflorescences, stems and leaves***

The data summarized in Table 1 show some interesting differences between the results of the HS-SPME analysis of inflorescences, stems and leaves of *T. argentina*:

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 (39 and 38 respectively), while the stems contribute a smaller number (only 25 compounds).

2) The main components *cis*- and *trans*-tagetenone were found at greater proportions in inflorescences and leaves than in stems. However, the stems were the main source of dihydrotagenone and β-caryophyllene.

3) Both *cis*-and *trans*-tagetone occurred at approximately the same concentrations in inflorescences, stems and leaves.

4) β-thujone, β-cadinene and neophytadiene were minor compounds and were only present in inflorescences. Moreover, p-cymene, 4-terpineol, *cis*-carvone, germancrene B and guaiol were exclusive components of the leaves whereas *cis*-β-ocimene was only observed in the stems.

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

In data reported in the literature (Zygadlo *et al*., 1993a), the components present in the essential oil were eleven, two of which were identified at trace level. However, using the HS-SPME analysis in the same previously established conditions, 41 components were measured on the aerial parts of the whole plant.

Comparing the results obtained for essential oil analysis reported in the literature and the HS-SPME data obtained here, revealed the majority of components to be the same. However, HS-SPME analyses showed a better characterization of the volatile organic compounds present in *T. argentina* than the analysis of essential oils, because it was possible to identify a greater number of minority components that perhaps could not be observed by essential oil analysis as they are lost during the hydrodistillation process.

**CONCLUSIONS**

A simple, rapid and solvent-free technique to determine the volatile components in *T. argentina* plants using the HS-SPME/GC-MS and GC-FID methods was established. Using a smaller amount of samples, a shorter extraction time and a much simpler procedure, the HS-SPME method can achieve at least comparable and often better results than those obtained by essential oil analysis.

The method developed provides a fast and easy characterization of the volatile compound profiles, which can therefore be used in further studies

aimed at characterizing different populations of *T. argentina* by HS-SPME analyses.

**Table 1**
Volatile compounds observed in *Tagetes argentina* Cabrera.

| Peak | Rt [a] (min) | Compound [b] | % [c] | | | | $KI_e$ [d] | $KI_r$ [e] |
|---|---|---|---|---|---|---|---|---|
| | | | I. [f] | L. [g] | S. [h] | W. P. [i] | | |
| 1 | 14.91 | *p*-cymene | - | 0.8 | - | - | 1025 | 1025 |
| 2 | 15.16 | limonene | 0.5 | 0.6 | - | 0.9 | 1032 | 1033 |
| 3 | 15.63 | dihydrotagetone | 5.2 | 2.8 | 29.6 | 6.4 | 1045 | 1047 |
| 4 | 15.92 | *cis*-β-ocimene | - | - | 0.3 | - | 1053 | 1054 |
| 5 | 16.13 | *trans*-β-ocimene | 3.0 | 0.6 | 3.3 | 2.6 | 1059 | 1058 |
| 6 | 16.96 | β-thujone | 0.3 | - | - | - | 1082 | 1081 |
| 7 | 17.28 | isoterpinolene | 0.5 | 0.4 | - | 0.8 | 1091 | 1091 |
| 8 | 17.38 | α-pyridone | 0.6 | 0.3 | - | 0.8 | 1094 | 1094 |
| 9 | 18.22 | chrysanthenone | 0.3 | 0.3 | - | 0.5 | 1119 | 1121 |
| 10 | 18.71 | *allo*-ocimene | 0.8 | 0.6 | 3.1 | 1.1 | 1134 | 1132 |
| 11 | 19.23 | *trans*-tagetone | 8.8 | 7.7 | 7.9 | 11.8 | 1150 | 1149 |
| 12 | 19.54 | *cis*-tagetone | 10.1 | 7.2 | 8.2 | 9.4 | 1159 | 1156 |
| 13 | 19.94 | p-cymen-8-ol | 2.0 | 1.5 | - | 1.7 | 1172 | 1172 |
| 14 | 20.06 | Unknown | - | - | - | 0.1 | 1176 | |
| 15 | 20.38 | 4-terpineol | - | 10.1 | - | 0.1 | 1185 | 1182 |
| 16 | 20.81 | carvone | 0.2 | 6.0 | - | 0.3 | 1198 | 1200 |
| 17 | 21.24 | verbenone | 1.3 | 1.1 | - | 1.1 | 1213 | 1218 |
| 18 | 21.34 | Unknown | 0.1 | - | - | 0.1 | 1216 | |
| 19 | 22.00 | *cis*-tagetenone | 29.6 | 33.3 | 4.1 | 17.9 | 1238 | 1232 |
| 20 | 22.46 | *trans*-tagetenone | 21.1 | 14.4 | 2.8 | 28.8 | 1253 | 1252 |
| 21 | 22.77 | Unknown | 0.1 | - | - | 0.1 | 1263 | |
| 22 | 22.91 | anisole | 1.1 | 0.4 | - | 0.7 | 1268 | 1265 |
| 23 | 23.44 | piperitone | 1.6 | 1.3 | - | 1.3 | 1285 | 1282 |
| 24 | 23.71 | Unknown | - | 0.1 | - | - | 1294 | |
| 25 | 24.74 | Unknown | 0.2 | - | - | 0.1 | 1328 | |
| 26 | 25.16 | methyleugenol | 0.4 | 0.2 | 0.7 | 0.5 | 1345 | 1348 |
| 27 | 25.38 | δ-elemene | 0.4 | 0.2 | 1.6 | 0.4 | 1352 | 1353 |
| 28 | 25.80 | *cis*-carvone oxide | - | 0.8 | - | - | 1367 | 1366 |
| 29 | 26.27 | Unknown | 0.1 | - | 0.3 | 0.1 | 1384 | |
| 30 | 26.47 | Unknown | 0.3 | - | 0.4 | 0.1 | 1391 | |
| 31 | 26.77 | β-longipinene | 0.6 | 0.4 | - | 0.6 | 1402 | 1403 |
| 32 | 27.56 | β-caryophyllene | 2.1 | 0.5 | 13.5 | 2.3 | 1432 | 1430 |
| 33 | 27.75 | Unknown | 0.2 | - | 0.3 | 0.1 | 1439 | |
| 34 | 27.94 | Unknown | 0.1 | - | - | 0.1 | 1446 | |
| 35 | 28.07 | aromadendrene | 1.1 | 1.5 | 2.4 | 1.0 | 1451 | 1452 |
| 36 | 28.15 | Unknown | - | - | - | 0.1 | 1454 | |
| 37 | 28.46 | calarene | 0.9 | - | 5.6 | 1.0 | 1465 | 1467 |
| 38 | 29.04 | α-amorphene | 0.3 | 0.9 | 0.8 | 0.3 | 1484 | 1485 |
| 39 | 29.03 | Unknown | - | 0.2 | - | - | 1487 | |
| 40 | 29.11 | Unknown | - | 0.1 | - | - | 1490 | |
| 41 | 29.22 | germacrene D | 2.4 | 1.2 | 4.5 | 2.5 | 1494 | 1492 |
| 42 | 29.38 | *trans*-α-farnesene | 0.1 | 0.6 | - | 0.1 | 1499 | 1500 |
| 43 | 29.63 | bicyclogermacrene | 2.9 | 1.2 | 5.4 | 3.0 | 1510 | 1511 |
| 44 | 29.80 | β-cadinene | 0.1 | - | - | 0.1 | 1517 | 1520 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 45 | 30.01 | γ-cadinene | 0.3 | 0.4 | 0.9 | 0.2 | 1525 | 1525 |
| 46 | 30.22 | δ-cadinene | 0.5 | 0.5 | 1.8 | 0.6 | 1533 | 1533 |
| 47 | 30.59 | spathulenol | 0.1 | - | 0.4 | 0.1 | 1548 | 1549 |
| 48 | 30.99 | germacrene B | - | 0.2 | - | - | 1564 | 1563 |
| 49 | 31.77 | guaiol | - | 0.5 | - | - | 1595 | 1598 |
| 50 | 35.78 | tetradecanoic acid | - | - | 0.4 | - | 1767 | 1765 |
| 51 | 37.43 | neophytadiene | 0.2 | 0.3 | 0.9 | 0.3 | 1839 | 1837 |
| 52 | 38.42 | Unknown | - | 0.1 | - | - | 1881 | |
| 53 | 40.85 | Unknown | - | 0.9 | 0.9 | - | 1968 | |
| Total | | | 100.0 | 100.0 | 100.0 | 100.0 | | |
| % Compounds Identified | | | 82.1 | 86.8 | 84.0 | 78.0 | | |
| % Area Identified | | | 99.5 | 99.7 | 99.0 | 99.1 | | |

[a] Rt: retention time; [b] Identified by GC-MS; [c] Quantified by GC-FID; [d] Experimental Kovat´s Retention Index; [e] Kovat´s Retention Index from the Literature; [f] Inflorescences; [g] Leaves; [h] Stems; [i] Whole Plant.

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