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Phytochemistry of *Tagetes minuta* L. (Asteraceae) from Córdoba, Argentina: Comparative study between essential oil and HS-SPME analyses

[Fitoquímica de *Tagetes minuta* L. (Asteraceae) originario de Córdoba, Argentina: estudio comparativo entre análisis del aceite esencial y 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 minuta* L. Five types of SPME commercial fibers including PA, PDMS, CAR-PDMS, PDMS–DVB and DVB–CAR–PDMS were investigated and the best extraction was achieved with the mixed fiber DVB–CAR–PDMS. Parameters for HS-SPME in terms of equilibrium time of HS, fiber exposition time and extraction temperature were also investigated. Additionally, the composition of inflorescences, leaves and stems was also studied separately by HS-SPME. As a result, 68 compounds were determined and 53 were identified. A comparison was made between results obtained by HS-SPME–GC–MS and steam distillation of essential oil of the aerial parts of the plant. In both analyses, the major components were: *cis*-tagetenone and *trans*-tagetenone. Using much smaller samples, a shorter extraction time and a simpler procedure, the HS-SPME method can achieve similar results to 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. minuta*.

Keywords: HS-SPME; essential oil; *Tagetes minuta*; volatile organic compounds; *Tagetes oil.*

Resumen

Se realizó el análisis de los componentes volátiles de *T. minuta* L. 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 estudiaron cinco tipos de fibras comerciales que incluyeron a PA, PDMS, CAR-PDMS, PDMS–DVB y DVB–CAR–PDMS y se estableció que la fibra de DVB–CAR–PDMS es la que posee mejor comportamiento en el proceso de extracción. Se determinó el efecto del tiempo de equilibrio del espacio de cabeza, de la temperatura de extracción y del tiempo de exposición de la fibra sobre el proceso de HS-SPME. Adicionalmente, también se estudio por separado la composición de inflorescencias, hojas y tallos empleando el método de HS-SPME. Como resultado de este estudio se determinaron 68 componentes de los cuales 53 fueron identificados. Por otra parte se realizó una comparación de los resultados HS-SPME con el análisis del aceite esencial obtenido de las partes aéreas de la planta. En ambos casos, los componentes mayoritarios fueron: *cis*-tagetenona y *trans*-tagetenona. Utilizando una muy pequeña cantidad de muestra, un corto periodo de tiempo y un procedimiento más simple se lograron similares resultados a los obtenidos mediante el análisis del aceite esencial. En conclusión, el método de HS-SPME desarrollado es simple, rápido, efectivo y libre de la utilización de solventes, puede ser fácilmente implementado para el análisis de componentes volátiles provenientes de muestras de diferentes poblaciones de *T. minuta*.

Palabras Clave: HS-SPME; aceite esencial; *Tagetes minuta*; compuestos orgánicos volátiles; aceite de *Tagetes*..

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List of Abbreviations*:*

- *T. minuta* – *Tapetes minuta* L.
- EO – essential oil
- HS-SPME – Headspace Solid Fase Microextraction
- GC – Gas Chromatography
- MS – Mass Spectrometry
- FID – Flame ionization Detector
- Rt – Retention time
- PDMS – polydimethylsiloxano
- CAR – carboxene
- DVB – divinylbencene
- PA – polyecrilate.

INTRODUCTION

Asteraceae is the largest family of vascular plants with more than 23,000 species (Jeffrey, 2007). The genus *Tagetes* belongs to this large family. The mostly American genus *Tagetes* (family Asteraceae) with more than 50 known species (Mc Vaugh, 1984) originated in Central and South America (Kaplan, 1958). Many of these are used medicinally. The antimicrobial activity of *T. lucida* has been examined (Cespedes *et al*., 2006); and in the particular case of Argentina is represented by 12 species, of which 5 are endemic (Ariza Espinar, 1967; Barboza *et al*, 2006).

Tagetes minuta L. is one of the three species found in the province of Córdoba (Argentina), growing wild from spring to early winter when it completes its life cycle. It is found in the mountainous area and is commonly known in our country as "Suico" or "Chinchilla". Although this plant is native to South America, after the Spanish conquest it was introduced in Europe, Asia, Africa and Australia (Babu and Kaul, 2007).

The essential oil of this plant, known commercially as "Tagetes oil", has applications in food production, including the preparation of alcoholic beverages, cola and frozen dairy desserts, as well as sweets, jellies, puddings and spices (Leung, 1980). In addition, this essential oil has also been described to have biological activity against certain pathogens (Tereschuk *et al*., 1997) and is well known for its biocidal properties (Kéïta *et al*., 2000, Wells *et al*. 1992; Gillij *et al*., 2008). Nowadays, Tagetes oil use as a raw material in the flavor and perfumery industry is widespread. (Gil *et al*., 2000).

The chemical composition of *Tagetes* essential oils has been widely investigated (Chalchat *et al*., 1995; Bansal *et al*., 1999; Gil *et al*., 2000; Prasad *et al*., 2002). Despite variations in their relative concentrations, the main constituents of *T. minuta* oils are limonene and *cis*-ocimene (monoterpenes), dihydrotagetone, *trans*- and *cis*-tagetone, *trans*- and *cis*-tagetenone (oxygenated monoterpenoids). Other oxygenated monoterpenoids (terpinolene, carvacrol, and carvone) and sesquiterpenes (β-caryophyllene, germacrene D, γ-elemene) have also been reported at lower concentrations.

Various isolation and instrumental techniques have been used to study the complex oils of *Tagetes*, but steam distillation and chromatography have been shown to be the most adequate techniques (Curtis, 1962, Downum, 1983). However, 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 sample. 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; 2004; 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) was introduced by Pawliszyn and co-workers (Arthur *et al*., 1990), with this technique proving increasingly useful in organic analytical chemistry because it is a rapid and simple procedure of extraction possessing a great capacity of concentration without the need for any organic solvent (Cai *et al*., 2006; Vas and Vékey, 2004). Headspace SPME permits the establishment of equilibrium between the sample matrix, the headspace above the sample, and a stationary phase coated on the

fused silica fiber. It has been used for the extraction of volatile compounds in various matrices, such as vegetables, fruits, juices, soft drinks or alcoholic beverages (Kataoka *et al*., 2000), and it has 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 is used to characterize the volatile fraction of aromatic and medicinal plants (Marriott, 2001; Smith, 2003), offering 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 SPME analysis of volatile constituents on whole plants of *T. minuta* or their aerial parts. For this reason, the present study describes HS-SPME/GC–MS and GC-FID methods for the analysis of volatile compounds in *T. minuta* and SPME optimal parameters in terms of fiber types, extraction temperature, equilibrium time and extraction time. Also a comparison between the results obtained by HS-SPME and essential oil analyses on the characteristic GC–MS and GC-FID profiles was performed for both qualitative and semi-quantitative analyses of volatile compounds from *T. minuta.*

MATERIALS AND METHODS

Plant Samples

Specimens of *T. minuta* L. in the process of flowering-fruiting were collected between March and April 2010 in the Sierras Grandes de Córdoba, Argentina. A whole plant was deposited in the Herbarium Marcelino Sayago (Register Number ACCOR 336b), 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 were placed in glass vials of 20 cm^3, which were sealed with Viton septa and aluminum seals provided by Supelco (Sigma-Aldrich, Argentina).

Essential oil of T. minuta

500 g of fresh aerial parts of plants were hydrodistilled for 3 hours. The aqueous distillate was extracted with chloroform (3 x 20 mL) and the organic layer was separated, dried over anhydrous $MgSO_4$ and filtered. The solvent was evaporated in a rotary evaporator (ambient temperature) to obtain 0.1 mL of essential oil (yield of 0.02% v/w).

Selection of extraction fiber

Five different commercial fibers were evaluated: PDMS 100 μm; PDMS-DVB 65 μm; CAR-PDMS 85 μm, DVB-CAR-PDMS 50/30 μm and PA 85 μm, all supplied by Supelco (Sigma-Aldrich of Argentina). Using a manual holder (Supelco), all fibers were conditioned in the GC injector at 225 °C for 8 hours before use. The vials containing the samples were immersed 10 mm in a thermostatic water bath at 30 °C (PolyScience 8005, accuracy ± 0.2 ºC). After 10 min, the SPME device was inserted into the sealed vial by manually penetrating the septum, and the fiber was exposed to the whole plant material headspace for 5 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

HS-SPME optimization

Having established which fiber had the highest affinity for the volatile components (greatest total area and greatest peak area of the main components in the chromatogram), tests aimed at discovering the optimum extraction temperature, equilibrium time and exposure time were performed. To carry this out, two of the parameters were maintained invariable while the other parameter was altered.

The tests to determine the optimum temperature of extraction were performed in the range 30 to 70º C. Those to determine the optimal equilibrium time were carried out in the range 10 and 60 min and the ones to determine the optimal time of exposure to HS were performed in the range 5 and 60 min.

Gas Chromatography (GC)

Analyses were performed using a gas chromatograph Shimadzu GC14B, equipped with a flame ionization detector, a manual injection port operating in 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: 225 °C; initial temperature: 40° C (5 min); final temperature: 200 °C (5 min); heating rate: 5 °C/min; detector: 230 °C; carrier gas: N_2 99.99% and head pressure: 5 psi. 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 volatile components was performed using a gas chromatograph HP 5890 Series II equipped with a manual injection port operating in a splitless mode and coupled to an HP 5970 Mass Detector. 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: 225 ºC; initial temperature: 40 ºC (5 min); final temperature: 200 ºC (5 min); heating rate: 5 °C/min; interface: 230° 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 (match ≥ 80) 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

Selection of extraction fiber

Although changes in the extraction conditions can strongly affect the total peak area, the number of chromatographic peaks is not influenced. Therefore in this study, the total peak area was used as a parameter (Mejías *et al*., 2002; Díaz *et al*., 2002) to optimize SPME extraction conditions. Additionally, the effect of these variables on the peak area of the main components was studied.

Figure 1 shows the effect of fiber type on the total peak area of the chromatogram and on the peak area of the main components. As can be seen, the order of extraction efficiency of the volatile components as determined by the total area of the chromatograms was: DVB-CAR-PDMS ≥ PDMS > DVB-PDMS > BP ≥ CAR-PDMS, with the fibers of DVB-CAR-PDMS and PDMS showing a 40% higher extraction efficiency than the other fibers tested.

DVB-CAR-PDMS and PDMS fibers had a very similar affinity for the volatile components of *T minuta*, although the fiber DVB-CAR-PDMS DVD presented a slightly higher capacity than that of PDMS.

Some differences were observed in the amount of principal components extracted. Dihydrotagetenone, anisole and bicyclogermacrene were extracted better by the PDMS fiber and verbenone whereas *cis* and *trans*-tagetenone were extracted at a slightly higher proportion by the CAR-PDMS DVD fiber. The extracted amount of *cis* and *trans*-tagetone were very similar in both fibers.

As shown in Figure 1, as DVB-CAR-PDMS was slightly more capable of extracting volatile compounds present in *T. minuta*, the fiber of DVB-CA - PDMS was finally adopted for the subsequent studies.

Selection of extraction temperature

Figure 2 shows the effect of extraction temperature on the total and main components areas observed in the chromatograms, where it can be observed that with increasing temperature there was a slight increase in the total area of the chromatogram peaks. Also, the extraction temperature affected other components: as extraction temperature increased so did the peaks areas of *cis* and *trans*-tagetenone whereas *cis* and *trans*-tagetone decreased. However, no significant effects were observed on the peak areas of dihydrotagetone, anisole verbenone or bicyclogermacrene.

In general, the extraction temperature has a significant influence on the HS-SPME because it affects the distribution coefficients of the volatile components from the sample matrix and the HS, and also between the HS and fiber. As shown in Figure 2, temperature had a different effect on each individual compound. The sharp increase in *cis* and *trans*-tagetenona with temperature showed that, from 60 ºC, the total area was mostly due to this compound. For this reason, increasing the extraction temperature had a negative effect on other components of the sample, perhaps because of a displacement effect caused by the higher affinity of the fiber for *cis* and *trans*-tagetenone. Therefore, we decided to perform the subsequent experiences at 40 ºC.

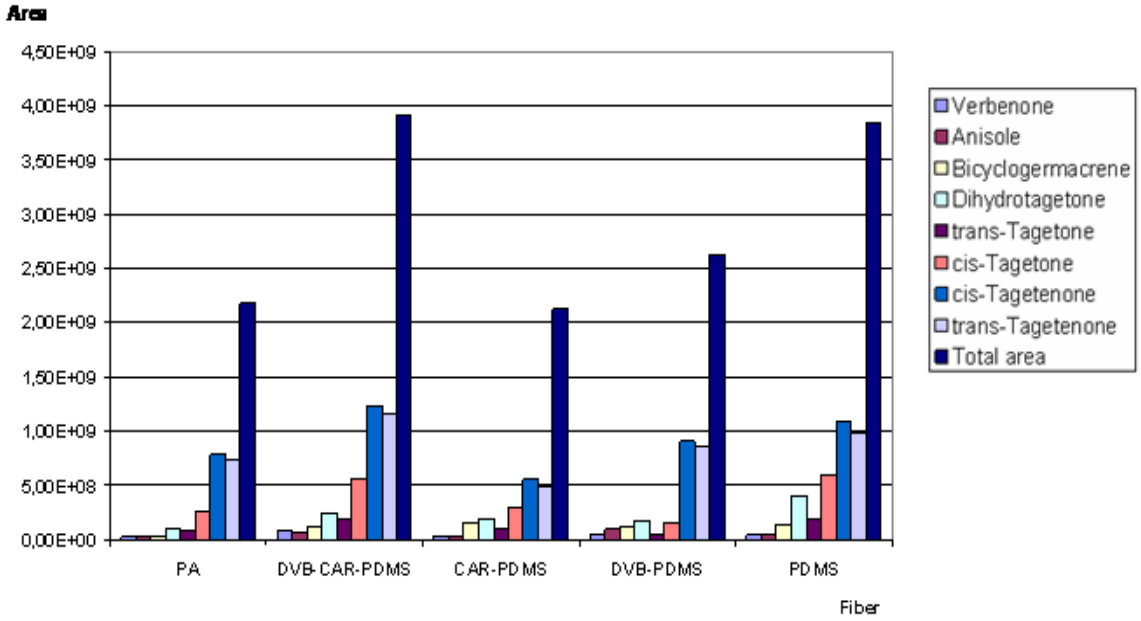

Figure 1. Effect of fiber type on the process of micorextraccion of the volatile components of *T. minuta* L

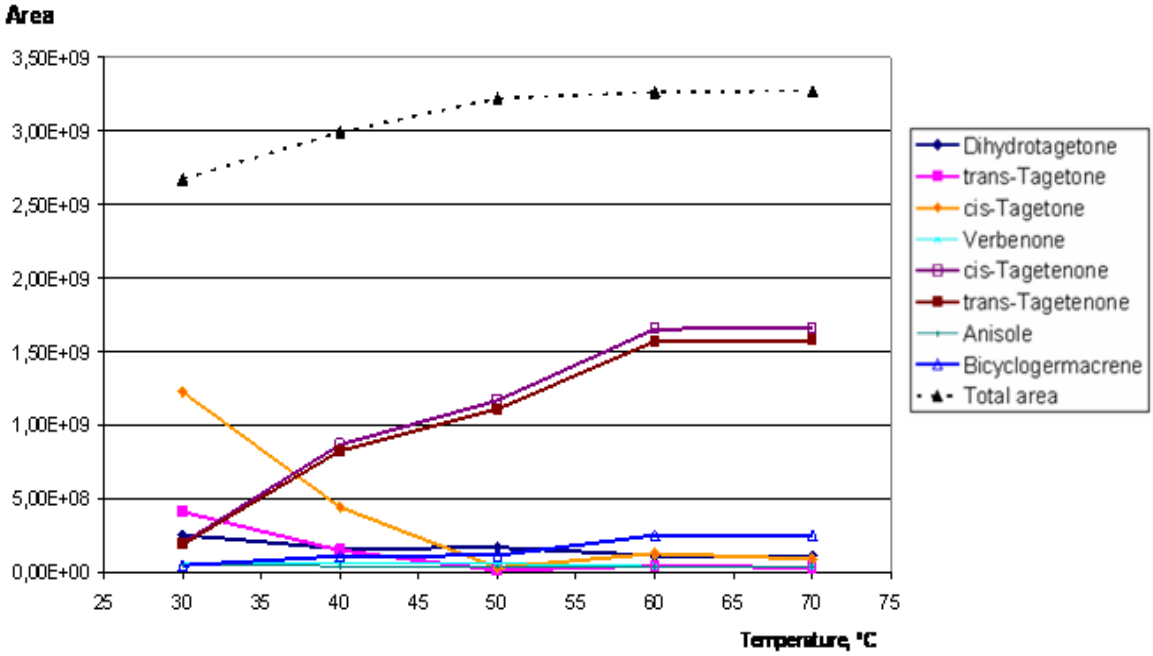

Figure 2. Effect of temperature on the microextraction of volatile components of *T. minuta* L.

Selection of equilibrium time

Figure 3 shows the effect of equilibrium time of HS on the total and main component peak areas of the chromatogram. As can be seen in this figure, the increase of equilibrium time of HS caused a slight increase of the total area and the area of the main

components for up to 10 min. After this time, the measured parameters remained constant. Thus, there was no significant effect of equilibrium time of HS on the SPME process, and for this reason we chose to work up to 10 min.

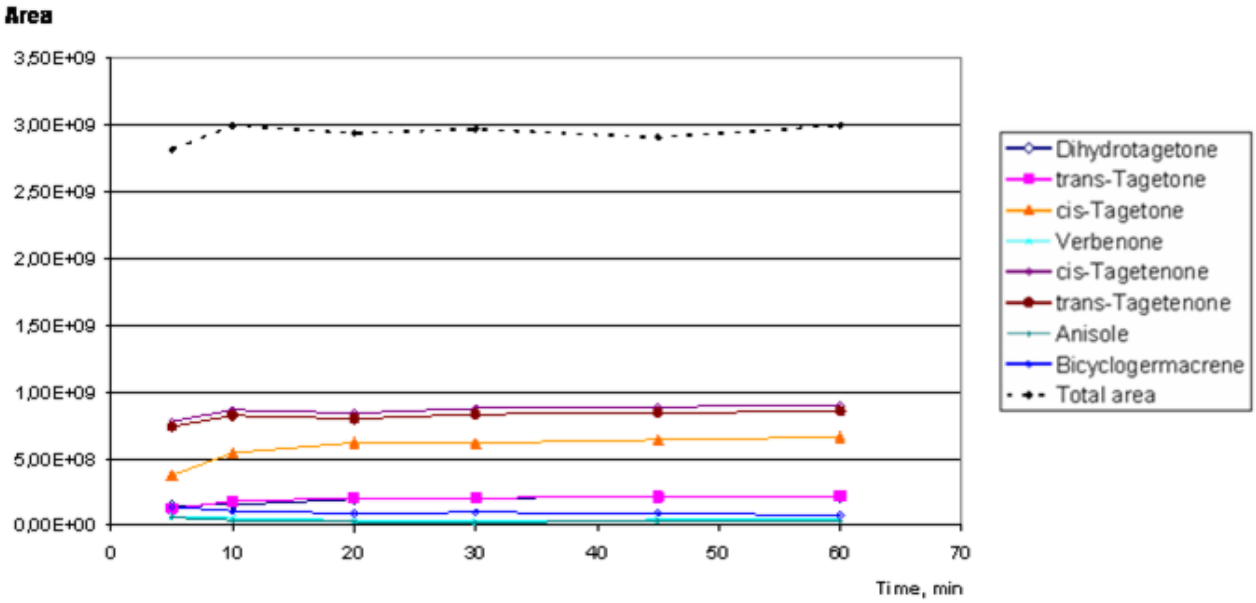

Figure 3. Effect of equilibrium time of HS on the microextraction of volatile components of *T. minuta* L

Selection of extraction time

Figure 4 shows the effect of exposure time of fiber to HS. It can be observed that as exposure time increased, there was a sharp rise in the total area of the peaks in the chromatogram, as well as for those corresponding to *cis* and *trans*-tagetenone. However, there was no significant effect on the other principal components. It was also established that the greatest response was observed at 30 min of fiber exposure.

It is noteworthy that the increase in the extraction time produced a greater effect on the exposure time of fiber to the HS than on the equilibrium time of the HS. Therefore, 40° C was adopted for the extraction temperature, 10 min for the equilibrium time and 30 min for the extraction time, as the HS-SPME parameters for the extraction of volatile compounds from fresh aerial parts of *T. minuta*.

HS-SPME of whole plant

Table 1 summarizes the main components provided by the HS-SPME analysis of the whole plant, with *cis*-tagetenone (15.4%) and *trans*-tagetenone (14.2%) being found at the greatest proportions.

In addition, there were also appreciable amounts of: tagetone (9.9%), anisole (7.9%), dihydrotagetone (5.6%), verbenone (5.0%), bycyclogermacrene (4.7%), *p*-cymen-8-ol (3.9%) and *trans*-pinocarvyl acetate (3.9%). The presence of β-caryophyllene (3.5%), *trans*-tagetone (3.3%), *allo*-ocimene (2.9%), piperitone (1.8%), δ-elemene (1.6%), α-pyridone (1.2%) and β-thujone (1.1%) was also determined, but to a lesser degree. The rest of the observed components were present at amounts ranging from 0.9% (filifolone) to 0.1% (for example cis-β-ocimene, among others).

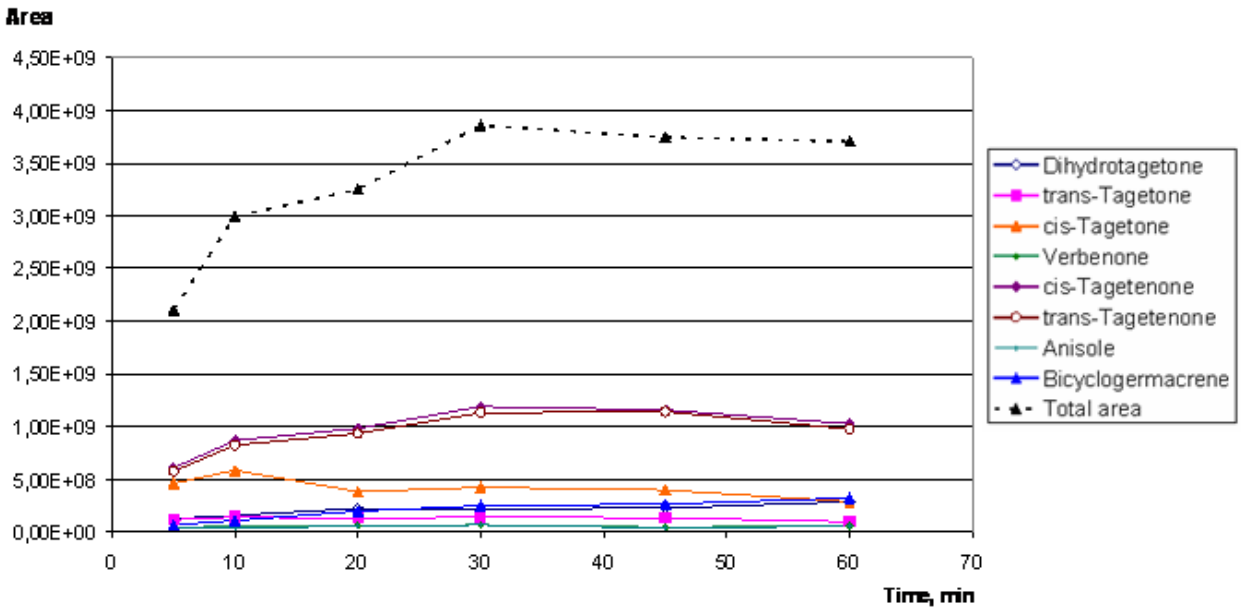

Figure 4. Effect of exposure time on the fiber microextraction of volatile components of *T. minuta* L.

HS-SPME of inflorescences

Trans-tagetenone (26.1%) and *cis*-tagetenone (17.8%) were established as major components by the results obtained on inflorescences (Table 1). Additionally, the presence of *cis*-tagetone (7.7%), verbenone (7.4%), dihyrotagetone (6.4%), *p*-cymen-8-ol (5.6%), *trans*-pinocarvyl acetate (4.2%), bicyclogermacrene (2.9%), *cis*-epoxycimene (2.6%), β-longipinene (2.6%), piperitone (1.5%) α-caryophyllene (1.3%), β-thujone (1.1%) and δ- elemene (1.1%) was determined but at smaller proportions. The rest of the observed components were present at amounts ranging from 0.9% (germacrene D) to 0.1% (for example γ-cadinene, among others).

HS-SPME of leaves

In the case of measurements made on leaves of *T. minuta* (Table 1), the major components were *cis*-tagetenone (29.0%) and *trans*-tagetenone (10.2%). Lower proportions were observed of *cis*-tagetone (7.9%), *trans*-tagetone (7.6%), bicyclogermacrene (6.8%), anisole (3.0%), *p*-cymen-8-ol (2.7%), germacrene D (2.7%), verbenone (2.6%), aromadendrene (2.4%), β-caryophyllene (2.3%), *trans*-pinocarvyl acetate (2.2%), filifolone (1.9%), dihydrotagetone (1.7%), methyleugenol (1.5%), β-longipinene (1.4%), *trans*-anethole (1.3%), β-thujone (1.1%), α-pyridone (1.1%), piperitenone (1.1%) and *allo*-ocimene (1.0%). The rest of the observed components were present at amounts ranging from 0.8% (for example crotononitrile, among others) to 0.1% (for example carvone, among others).

HS-SPME of stems

On stems of *T. minuta* (Table 1), bicyclogermacrene (16.1%) and dihydrotagetone (15.5%) were observed as the main components. At smaller proportions we observed: α-caryophyllene (9.8%), calarene (8.6%), α-copaene (7.3%), aromadendrene (6.2%), germacrene D (4.8%), methyleugenol (3.6%), *cis*-epoxycimene (3.4%), β-caryophyllene (3.3%), δ-elemene (2.9%), *cis*-tagetone (1.6%), δ-cadinene (1.6%), β-longipinene (1.4%), β-elemene (1.1 %) and *cis*-tagetenone (1.0%). The rest of the observed components were present at amounts ranging from 0.8% (guaiol) to 0.2% (spathulenol).

Essential oil analysis

As can be seen in Table 1, the major components present in the essential oil of *T. minuta* were *trans*-tagetenone (32.3%) and *cis*-tagetenone (20.9%). There were also significant amounts of: dihydrotagetone (9.7%), *trans*-pinocarvyl acetate (7.1%) and carvone (4.3%). In addition, limonene (3.2%), *trans*-β-ocimene (2.8%), menthofuran (2.1%), *trans*-tategone (1.9%), piperitone (1.5 %), *p*-cymen-8-ol (1.4%), anisole

(1.4%) and α-terpinolene (1.1%) were also part of the volatiles present, but to a lesser extent. Other components were found at amounts ranging from 0.7% (*trans*-α-farnesene) to 0.02% (bicyclogermacrene).

Table 1. Volatile compounds observed in *T. minuta* L.

Peak	Rt [a] (min)	Compound [b]	% [c]					KI_e [d]	KI_r [e]
			I. [f]	L. [g]	S. [h]	W. P. [i]	E.O. [j]		
1	8,68	crotonitrile	-	0,8	-	-	0,4	664	664
2	13,00	sabinene	-	-	-	-	0,5	974	974
3	13,73	myrcene	-	-	-	-	0,1	993	993
4	15,16	limonene	0,2	0,7	0,6	0,5	3,2	1032	1033
5	15,71	dihydrotagetone	6,4	1,7	15,4	5,6	9,7	1047	1047
6	15,75	phenylacetaldehyde	-	-	-	-	0,1	1049	1049
7	15,93	*cis*-β-ocimene	0,2	-	0,6	0,1	0,3	1054	1054
8	16,10	*trans*-β-ocimene	0,3	0,6	-	0,6	2,8	1058	1058
9	16,96	β-thujone	1,1	1,1	-	1,1	0,5	1082	1081
10	17,40	α-pyridone	0,6	1,1	-	1,2	0,1	1095	1094
11	17,71	α-terpinolene	-	-	-	-	1,1	1104	1100
12	17,90	filifolone	0,6	1,9	-	0,9	0,1	1109	1083
13	18,14	Unknown	-	-	-	-	0,2	1117	
14	18,23	chrysanthenone	0,8	0,8	-	0,7	0,1	1120	1121
15	18,38	Unknown	-	0,1	-	-	0,1	1124	
16	18,52	Unknown	-	-	-	-	0,2	1128	
17	18,62	*allo*-ocimene	0,8	1,0	-	2,9	0,2	1131	1132
18	18,72	*cis*-epoxyocimene	2,6	0,8	3,4	0,5	0,6	1135	1139
19	19,09	*trans*-epoxyocimene	1,3	0,3	-	0,2	0,1	1146	1141
20	19,27	*trans*-tagetone	0,8	7,6	0,4	3,3	1,9	1151	1149
21	19,65	*cis*-tagetone	7,7	7,9	1,6	9,9	0,2	1163	1156
22	19,99	*p*-cymen-8-ol	5,6	2,7	-	3,9	1,4	1173	1172
23	20,29	4-terpineol	-	-	-	-	0,2	1183	1182
24	20,70	Unknown	-	-	-	-	0,8	1195	
25	20,93	carvone	0,6	0,1	-	0,5	4,3	1202	1200
26	21,18	menthofuran	-	-	-	-	2,1	1211	1187
27	21,33	verbenone	7,4	2,6	-	5,0	0,3	1216	1218
28	21,59	2-phenyl-1,3-dioxolane	-	-	-	-	0,5	1224	1215
29	21,81	*cis*-tagetenone	17,8	29,0	1,0	15,4	20,9	1231	1232
30	22,52	*trans*-tagetenone	26,1	10,2	3,0	14,2	32,3	1255	1252
31	22,73	Unknown	-	-	-	-	0,4	1262	
32	22,92	anisole	-	3,0	-	7,9	1,4	1268	1265
33	23,24	Unknown	-	-	-	-	0,2	1279	
34	23,36	piperitone	1,5	1,1	0,4	1,8	1,5	1283	1282
35	23,86	*trans*-pinocarvyl acetate	4,2	2,2	-	3,9	7,1	1299	1298
36	23,99	*trans*-anethole	0,5	1,3	0,8	0,5	0,1	1304	1301
37	24,19	piperitenone	0,3	0,1	-	0,3	0,1	1311	1310
38	24,80	Unknown	-	0,2	-	0,4	-	1332	
39	24,90	eugenol	0,5	0,2	0,4	0,1	-	1336	1337
40	25,27	methyleugenol	0,7	1,5	3,6	1,1	0,1	1349	1348
41	25,49	δ-elemene	1,1	0,4	2,9	1,6	-	1357	1353
42	25,58	α-cubebene	0,1	0,2	0,3	0,1	0,1	1360	1358
43	26,20	Unknown	-	0,1	-	-	0,4	1382	
44	26,35	Unknown	0,1	-	0,4	0,1	-	1387	

45	26,57	α-copaene	0,2	0,1	7,3	0,3	-	1395	1392
46	26,89	β-longipinene	2,6	1,4	1,4	2,0	0,4	1406	1403
47	27,11	Unknown	0,1	-	-	-	-	1415	
48	27,20	β-elemene	-	-	1,1	-	-	1418	1420
49	27,54	β-caryophyllene	0,6	2,3	3,3	3,5	0,3	1431	1430
50	27,61	α-caryophyllene	1,3	0,3	9,8	-	-	1433	1433
51	27,92	Unknown	0,1	0,3	-	0,1	0,1	1445	
52	28,11	aromadendrene	0,6	2,4	6,2	0,2	0,1	1452	1452
53	28,19	Unknown	0,1	-	0,5	0,9	0,1	1455	
54	28,50	calarene	0,6	0,4	8,6	0,1	0,2	1467	1467
55	28,59	Unknown	-	-	0,3	1,6	-	1470	
56	28,66	Unknown	-	0,3	0,4	-	-	1473	
57	29,06	α-amorphene	0,1	0,6	0,7	0,1	-	1488	1485
58	29,22	germacrene D	0,9	2,7	4,8	1,9	0,3	1494	1492
59	29,42	*trans*-α-farnesene	-	0,2	0,5	-	0,7	1502	1502
60	29,67	bicyclogermacrene	2,9	6,8	16,1	4,7	0,02	1512	1511
61	29,88	β-cadinene	-	-	0,6	-	-	1520	1520
62	30,03	γ-cadinene	0,1	0,3	0,6	0,1	0,1	1526	1525
63	30,22	δ-cadinene	0,1	0,5	1,6	0,3	0,1	1533	1533
64	30,59	spathulenol	-	0,1	0,2	-	-	1548	1549
65	31,65	Unknown	-	0,1	-	-	0,3	1590	
66	31,84	guaiol	-	-	0,8	-	0,2	1598	1598
67	33,23	α-cadinol	-	-	0,4	-	-	1656	1656
68	37,43	neophytadiene	-	0,3	-	-	0,2	1839	1837
		Total	100	100	100	100	100		
		% Compounds Identified	90.2	87.2	88.6	88.1	81.5		
		% Area Identified	99,8	99,0	98,4	96,8	97,2		

HS-SPME analysis vs. essential oil analysis

As can be seen in Table 1, we established the existence of 68 different components in the volatile fraction of *T. minuta*, 53 of which were successfully identified.

In the essential oil, 54 components were quantified (Table 1), 44 of which (81.5%) were identified. Thus, positive identification was achieved in 97.2% of the total area observed in the chromatogram.

Using the HS-SPME analysis in the previously established conditions, 42 components were measured on the aerial parts of the whole plant, achieving a positive identification of 37 components (88.1%). Thus, the total identified products represent 96.8% of the total area observed in the chromatogram.

Additionally, on studying different parts of the plant separately by HS-SPME: the inflorescence presented 41 different compounds, of which 37 (90.2%) were identified; 47 components were observed on the leaves, of which 41 (87.2%) were identified; 35 compounds were also observed on stems, of which 31 (88.6%) were identified. This implies a positive identification of 99.8%, 99.0% and 98.4%, respectively, of the total area of chromatograms.

The data summarized in Table 1 show some interesting differences between the results of the HS-SPME analysis of the whole plant and those from the essential oil:

1) Comparing the essential oil and HS-SPME data revealed most of the components to be the same. However, the percentages were reversed. Whereas *trans*-tagetenone was the main component in the essential oil, in HS-SPME *cis*-tagetenone prevailed (Table 1).

2) The amount of bicyclogermacrene was 235 times higher in HS-SPME than in essential oil. This may have been due to a high affinity of the fiber that was able to preconcentrate the compound. However, further studies are needed to test this hypothesis.

3) At short retention times (8 - 24 min), there was a greater number of minor components in the essential oil than in HS-SPME. At retention times ranging from 24 to 29 min using HS-SPME, minority components were frequent.

Moreover, the analysis of the aerial parts of *T. minuta* using the HS-SPME technique separately showed that:

1) *cis*- and *trans*-tagetenone were found predominantly in inflorescences and leaves, while the proportion in the stem was low.

2) dihydrotagetone, bicyclogermacrene, α-copaene, methyleugenol, α- and β-caryophyllene, germacrene D and calarene were components predominantly located in the stems. However, dihidrotagetone was found at a greater proportion in the inflorescences, and bicyclogermacrene was encountered at higher amounts in the leaf.

3) *cis*-tagetone predominated in leaves while similar amounts of *trans*-tagetone were found in inflorescences and leaves.

4) *p*-cymen-8-ol and verbenone predominated in inflorescences and leaves, but were not observed in the stems.

5) anisole, crotonitrile, neophytadiene were found exclusively in leaves.

6) β-elemene, β-cadinene, guaiol, α-cadinol were only detected as components of stems.

CONCLUSIONS

A simple, rapid and solvent-free technique to determine the volatile components in *T. minuta* plants using the HS-SPME-GC–MS and GC/FID methods was developed. Differences between the essential oil profiles and the data obtained by HS-SPME were compared. Using smaller samples, a shorter extraction time and a much simpler procedure, the HS-SPME method can achieve comparable results to those obtained by essential oil analysis.

The developed method provides a fast and easy characterization of the volatile compound profiles, which can be used in further studies aimed at characterizing different populations of *T. minuta* by HS-SPME.

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