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ARTICLE

Identification of Odor Impact Compounds of *Tagetes minuta* L. Essential Oil: Comparison of Two GC-Olfactometry Methods

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Odor impact compounds of *Tagetes minuta* L. essential oil were studied by gas chromatography (GC)-olfactometry using aroma extract dilution analysis (AEDA) and vocabulary-intensity-duration of elementary odors by sniffing (VIDEO-Sniff). AEDA was conducted by direct injection and revealed the presence of 43 odorant zones. Highest flavor dilution (*FD*) values were obtained for ethyl 2-methylpropanoate, ethyl 3-methylbutanoate, (*E*)-ocimenone, two tentatively identified thiols, and two yet unknown compounds. VIDEO-Sniff was realized by dynamic headspace sampling (D-HS) combined with 8W-GC-olfactometry where eight sniffers simultaneously detect volatile compounds obtained from a single chromatographic separation and revealed the presence of 42 odorant zones. Odorant trace compounds detected by GC-O that were present in quantities inferior to the GC-qMS system's detection limit and those subject to coelutions were identified by GC×GC-time-of-flight mass spectrometry (TOFMS). A total amount of 37 odorant components could be identified by VIDEO-Sniff, and the strong influence of the fruity notes of numerous esters stood out. Highest olfactory signals were obtained for ethyl 2-methylpropanoate, ethyl 2- and 3-methylbutanoate, and oct-1-en-3-one. Both methods hence come to the conclusion that ethyl 2-methylpropanoate and ethyl 2- and 3-methylbutanoate are among the main odorants in *Tagetes minuta* L. essential oil. Differences, advantages, and drawbacks of both GC-O methods are discussed.

KEYWORDS: GC-olfactometry; VIDEO-Sniff; 8W-GC-O; AEDA; *Tagetes*

INTRODUCTION

GC-Olfactometry (GC-O) uses the human nose as a detection device, generally parallel to a physical detector (i.e., a flame ionization detector, FID, or a mass spectrometer, MS) and thus permits rapid identification of so-called odorant zones in a chromatogram (*1*). By applying GC-O methodology, information on the olfactory impact of compounds in a sample can be obtained. These methods are generally divided into three groups: detection frequency methods (nasal impact frequency (NIF) and surface nasal impact frequency (SNIF)), direct time-intensity methods (odor specific magnitude estimation (OSME), finger span cross matching method (FSCM), and posterior intensity), and dilution to threshold methods (aroma extract dilution analysis (AEDA), combined hedonic of aromatic response measurement (CHARM)) (*1*). Dilution to threshold methods and especially AEDA are often used for their simplicity and uncomplicated data processing. One of the limitations of this method is the yet questionable necessity of only two panelists (sniffers or judges), but this factor can be controlled by realizing aroma recombination experiments with the obtained GC-O data. AEDA reveals the relative odor impact of the mixture's constituents (*2*). The sample is subsequently diluted by a factor of $d=2$ or 3 until an odor is no longer perceived. The panelist assigns odor description and presence/absence of perception. *FD* (flavor dilution) factors are calculated according to the following equation: $FD = d^{n-1}$, where d is the dilution factor (in this case $d=3$, constant) and n the number of dilution necessary for the odors to be no longer perceived (*1*). Dilution to threshold analyses are time-consuming due to the numerous dilution steps, and individual responses vary between judges. This is why the use of only two panelists is questionable, and a panel of at least eight judges is strongly recommended for GC-O (*3*). Time-intensity methods (TIM) and frequency of detection methods use a panel of 8 to 12 judges. These methods measure the odorant compounds' intensity (TIM) or the number of panelists able to detect a given odor in one single sample (detection frequency) without any dilution steps. They are thus not based on individual detection thresholds, and their aim is to detect all odorant compounds present in the given sample (*1*). The VIDEO-Sniff method (vocabulary-intensity-duration of elementary odors by sniffing), developed by the French National Agronomy Research Institute (INRA), is a hybrid approach

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which combines detection frequency and time-intensity methods. This method also includes the vocabulary used by the sniffers to describe their odorant perceptions. By sorting the vocabulary in olfactory classes, an aromagram by olfactory classes is obtained where each olfactory signal is assigned to at least one class having a specific color. Hence, visual interpretation of the aromagram becomes possible (*4*). Several acquisitions are needed in order to obtain reliable results, and in order to limit analyses time, the INRA has developed a fully computerized eight-port GC-O system (8W-GC-O) that allows for individual aromagrams of a panel of eight judges to be obtained simultaneously and in one analysis (**Figure 1**) (*5*,*6*). The system is designed to synchronously and evenly distribute volatile compounds separated by chromatography to eight sniffing ports. Thus, eight sniffers can simultaneously detect odorants eluting in a single chromatographic separation and in exactly identical conditions. Individual aromagrams are subsequently summed up to give the total olfactometric signal (TOS) using the AcquiSniff software according to the VIDEO-Sniff method (*7*). Odorant compounds are identified by GC-O-MS. In some cases, odorant trace compounds are present in quantities inferior to the qMS system's detection limit, or they coelute with major compounds. One possible solution for identification is the use of comprehensive two-dimensional GC. GC×GC uses two columns with different stationary phases (typically, non polar and polar) that are connected in series, hence resolving coelutions that occur in a conventional 1DGC system but also considerably increasing sensitivity. The combination of GC×GC with a MS-time-of-flight (TOFMS) detector provides a powerful and sensitive tool. However, in order to extract pertinent information, the data should be coupled to other information, such as GC-O results. Hence, the combination of GC-O with GC×GC, even when conducted on different instruments, allows the identification of numerous trace odorant compounds (*8*−*11*).

The use of AEDA and VIDEO-Sniff in 8W-GC-O mode on one sample gives information on the relative odor impact of the constituents in the extract (AEDA) but also detects a maximum number of odorants in one single run due to the use of a sniffer panel and the 8W-mode (VIDEO-Sniff). When combining the information obtained by GC-O with GC×GC-TOFMS experiments, odor impact trace compounds can be identified.

The matrix chosen for the experiments was *Tagetes minuta* L. essential oil. *T. minuta* L. is an angiosperm (flowering plant) belonging to the botanical order *Asterales*, family *Asteraceae*, genus *Tagetes*, species *minuta* (*12*). It is an annual herb which grows up to a height of two meters. The name *Tagetes* comes from Tages, an Etruscan god, and the plant's common name is marigold. Numerous synonyms are used such as *T. bonariensis* Pers., *T. glandulifera* Schrank, *T. glandulosa* Schrank ex Link, porchyllum Vell., and *T. riojana* Ferraro. The plant is cultivated for its essential oil, which is marketed under the name *Tagetes* oil. It has a characteristic odor described as sweet−sour, fruity−floral, fruity, sage, honey, with an eucalyptus and slightly rotten fruit note (LCMBA, unpublished results). It is used by flavor, fragrance, and food industries for drinks, frozen desserts, candy, and fine perfumery (*13*, *14*). Its chemical composition is a complex mixture of volatile terpenoids and has been exhaustively studied in the past by numerous research teams. Major compounds are limonene, (*Z*)-*β*-ocimene, dihydrotagetone, (*E*)- and (*Z*)-tagetone, (*E*)- and (*Z*)-ocimenone, and alloocimene. However, we found that the only monoterpenoid with a high *FD* factor is (*E*)-ocimenone. According to our literature studies, to date about 220 compounds have already been identified in *T. minuta* essential oil, but there is no information available on the components contributing to the essential oil's characteristic odor (*13*, *15*−*18*). *Tagetes* plants are also known for the presence of phototoxic and nematocidic thiophenes (*19*−*26*).

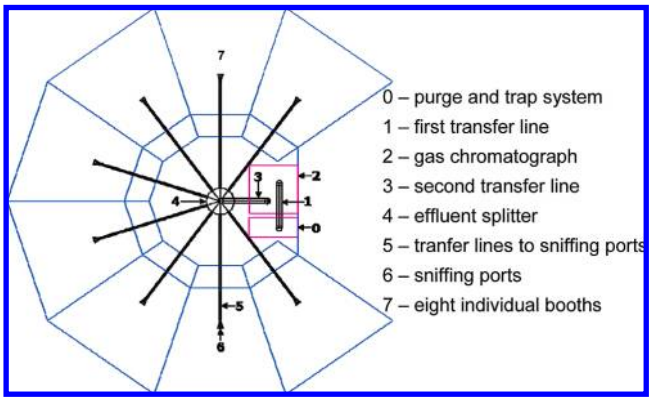

Figure 1. Architecture and overview of the 8W-GC-O device. The configuration presented is coupled to a system of extraction−concentration of volatile components of the purge-and-trap type. The setup consists of eight individual booths each with their own lighting, soundproofing, and laminar flow conditioned air (deodorized by filtering trough active carbon and acclimatized at 22 °C). The air circulates from top to bottom so as to isolate the sniffing zone as much as possible from olfactory interference by ambient air or the sniffers' body odor. The diameter of the whole system is 4.5 m.

To date and to the authors' knowledge, there is no publication on the identification of odorant volatiles and GC-Olfactometry studies of *Tagetes* plants. We report here the first study of odor impact compounds of *T. minuta* L. essential oil.

MATERIALS AND METHODS

Chemicals and Extracts. Commercial *T. minuta* L. essential oil (EO) was provided by Robertet S.A., France. The oil was obtained by hydrodistillation of the aerial parts (flowers, leaves, and stems) harvested in South Africa from March 6th to March 16th, 2007.

Analytical Studies. *GC-O/AEDA and Identification*

GC-Olfactometry (AEDA). GC-O/AEDA analyses were performed on a Shimadzu GC-2010 GC (Shimadzu, Champs-sur-Marne, France) equipped with an automatic injector type AOC-20i, a FID, and an ATAS olfactory port OP275 with a glass nasal cone (ATAS, Veldhoven, Netherlands). Samples were analyzed on a fused-silica capillary column: DB-1 (50 m length × 0.32 mm internal diameter × 0.52 μm film thickness; J&W, Folsom, USA). Carrier gas, nitrogen; constant pressure, 80 kPa; injector temperature, 250 °C; detector temperature, 250 °C; splitless mode, splitless time 1 min; purge flow, 100 mL/min. Since the nose is very sensitive to odors and in order to increase chances for finding peaks in the chromatogram corresponding to the smelled odors, 60% of the flow was directed to the FID, while 40% was directed into the heated sniffing port. Capillary transfer line leading to the nasal cone, 0.25 mm i.d.; total length, 1.50 m; length outside the oven, 1.49 m; temperature, 250 °C. Capillary transfer line leading to the FID, 0.25 mm i.d.; length, 1.50 m. Temperature program: 40 to 130 at 2 °C/min, from 130 to 250 at 4 °C/min, then held isothermal for 50 min. Samples were diluted at 5% in diethyl ether and subsequently diluted by a factor 3. Lower dilution was tried but abandoned due to (a) too strong of an odor intensity, (b) tailing of major compounds due to column overload, and (c) it would also have prolonged total analysis time. Analysis was conducted by two nonsmoker panelists with no known anosmia, trained according to J.N. Jaubert's Field of Odors and used in the organoleptic evaluation of natural extracts as well as in GC-Olfactometry (*27*). Training was realized by first smelling standard compounds classified in odorant poles according to the Field of Odors in a static evaluation and then by realizing GC-O/AEDA analyses on a standard mixture of 12 odorants in order to evaluate panelist performance and to exclude specific anosmia. Sniffing sessions lasted 30 min in order to prevent fatigue. Four dilutions were necessary for no odor to be perceived anymore. The ATAS Olfactory Voicegram software was used for data acquisition, and data were processed with Microsoft Excel (*28*).

GC-MS. GC-MS analysis was carried out using a 5890 series II chromatograph coupled to a 5971A MS (Agilent, Massy, France). Samples were analyzed on a fused-silica capillary column HP-1 (polydimethylsiloxane, 50 m length × 0.20 mm internal diameter × 0.33 μm film thickness; Interchim, Montluçon, France) and HP-20 M (polyethyleneglycol, 50 m length × 0.20 mm internal diameter × 0.10 μm film thickness; Interchim, Montluçon, France). Carrier gas, helium; constant pressure, 220 kPa; injector temperature, 250 °C (apolar column) or 230 °C (polar column); split ratio, 1:100; temperature program, 60 to 250 °C at 2 °C/min then held isothermal (20 min) at 250 °C (apolar column) or 220 °C (polar column); ion source temperature, 155 °C; transfer line temperature, 250 °C (apolar column) or 230 °C (polar column); ionization energy, 70 eV; electron ionization mass spectra were acquired over the mass range 35−400 u.

Odorant Compound Identification. Identification of the constituents was based on matching against odor databases, linear retention indices (*LRI*), and computer matching of the corresponding mass spectrum against commercial libraries (Wiley6N, MassFinder 2.1 Library, NIST98), laboratory mass spectra libraries built up from pure substances on an apolar column (*29*−*31*). *LRI* were calculated after injection of a series of linear alkanes C6−C26 on apolar and polar columns in the same operating conditions. Compounds available in the laboratory were confirmed by reference compound injection. Odorant compounds were identified by attributing them to a signal in the FID chromatogram (if possible), *LRI*, and odor descriptors according to a laboratory-made GC-O database. Identification was confirmed by injection of the reference compound in GC-O (odor comparison) and GC-MS.

Internal Calibration. Internal calibration was carried out on an Agilent 6890 chromatograph equipped with a FID and an automatic injector (Agilent, Massy, France). Fused-silica capillary column, HP-1 (polydimethylsiloxane, 50 m × 0.20 mm i.d. × 0.33 μm film thickness; Interchim, Montluçon, France); carrier gas, nitrogen; constant flow, 1 mL/min; injector temperature, 250 °C; detector temperature, 250 °C; split ratio, 1/10; temperature program, raised from 60 to 250 °C at 2 °C/min and then held isothermal (50 min). Hexadecane (1000 ppm (v/v)) was added as IS (internal standard) to the EO prior to analysis. The FID response factors for compounds relative to hexadecane were taken as one. Hexadecane was used as IS for several reasons: (a) it elutes in an empty chromatographical zone and hence does not coelute with other compounds; (b) it is of medium volatility (bp 287 °C; *LRI* = 1600/1600) and thus elutes quite in the middle of the chromatogram; and (c) it was, according to a preliminary study of the EO without internal standard addition, not naturally present in the EO.

8W-GC-O/VIDEO-Sniff and Identification

Eight-Way Gas Chromatography−Olfactometry Device (8W-GC-O). The 8W-GC-O device used is described in Berdagué et al., and its architecture is shown in **Figure 1** (*4*−*6*).

Extraction, Concentration, and Injection. The volatile components of *T. minuta* essential oil were extracted by D-HS (Tekmar, Cincinnati, OH, USA). One microliter of EO was placed on glass wool at the bottom of a Pyrex extractor (ref M3, Mallières, Aubière, France). Extraction conditions were optimized as follows: the extractor was maintained at 40 °C and purged for 7.5 min with a helium stream at a flow rate of 10 mL/min (Messer, He/U purity: 99.995%). The trap (working length, 180 mm; inside diameter, 1/4 in. (1 in. = 2.54 cm); packed with Tenax TA 60−80 mesh adsorbent (Supelco, Bellefonte, PA, USA)) was operated at 40 °C. The dry purge step was set at 3 min to eliminate as much water as possible while minimizing the loss of volatile fraction (*32*). The volatile components were then desorbed from the trap at 180 °C for 10 min using helium (Messer, He/N55 purity: 99.9995%) and sent through the first transfer line (part 1, **Figure 1**) into the cryo-focalization zone (cooled at −150 °C with liquid nitrogen). The construction of the introduction system is described in Berdagué et al. (*4*). Splitless injection was used by heating the cryo-concentration zone to 220 °C in 10 s.

Separation. Volatile components were separated in an Agilent 4890D chromatograph (Agilent, Massy, France) on an RTX-5 fused-silica capillary column (5% diphenyl-, 95% dimethylpolysiloxane, 60 m length × 0.53 mm internal diameter × 1.50 μm film thickness; Restek, Evry, France). Temperature was programmed as follows: 5 min isothermal at 40 °C, 4 °C/min rise to 205 °C, and 5 min isothermal at 205 °C. Carrier gas, helium; constant flow, 8 mL/min. A second transfer line heated to 210 °C (part 3, **Figure 1**) connected the column to the effluent divider. Division and transfer to the eight sniffing ports were done according to the literature (*4*).

Identification of Odor-Active Compounds. An independent single GC-O-MS setup composed of an Agilent chromatograph 6890 (Agilent, Massy, France), a mass detector MSD5973 (Agilent, Massy, France), and a sniffing port were used according to the literature (*4*). The GC-O data from the multiport system were synchronized with those from the single port GC-O-MS setup by using the AcquiSniff software and the mass spectrometry software MSDChem Agilent C.00.00 (*4*, *33*, *34*). Volatile components were identified by computer matching against spectral databases (*29*−*31*), *LRI* (*31*,*35*), and odors (homemade database, LCBMA, Université de Nice-Sophia Antipolis, France, unpublished work). Injection of the reference compounds was done in order to confirm identification.

Acquisition and Analysis of Olfactometric Data. Data were acquired using the AcquiSniff software at a rate of 1 scan/s. During the sniffing sessions, the sniffers were instructed to (a) signal each odor perceived by pressing a push button for as long as the odor lasted, (b) to describe the odors orally, and (c) to quantify their intensity on a five-point scale (*4*). All sniffers were trained to use the equipment and were selected as nonsmokers without any known pathology for their sensitivity and ability to detect and consistently describe a wide range of odors during GC-O tests. To describe the odors, the sniffers could use a free choice of vocabulary that was to remain as simple and precise as possible. The sniffers had no information on the nature of the sample and were remunerated for their participation. Sniffing sessions lasted between 30−35 min, and since odorant zones were detected after t_r = 35 min, data were collected in two steps in order to avoid sniffer fatigue: a first run for compounds eluting between 2 and 35 min followed by a second run going from 25 to 55 min due to the complexity of the aromagram at retention times of about 30 min.

A numerical recording of vocabulary items, intensity, and persistence of odors was launched at the start of injection of the volatile compounds into the chromatographic column. Thus, eight aromagrams were obtained, and eight individual digital audio recordings were read after each session in order to incorporate their content into the aromagrams using the AcquiSniff software. The data were analyzed using the VIDEO-Sniff method by breaking the total olfactory signal (TOS, sum of the eight individual aromagrams) up into olfactory classes which were defined during data processing in order to highlight the key-odor zones of an aromagram (Table 2) (*4*, *6*, *7*, *36*).

Headspace Solid Phase Micro Extraction: Two-Dimensional Gas Chromatography−Time-of-Flight−Mass spectrometry (HS-SPME-2DGC-TOFMS). A SPME 75 μm fiber (Carboxen/PDMS) purchased from Supelco (Bellefonte, PA, USA) was used for the extraction of volatiles from the EO headspace. HS-SPME was used in order to work in conditions comparable to those of D-HS. The fiber was conditioned according to manufacturer recommendations prior to analysis. One microliter of EO was placed in a sealed 20 mL SPME vial. After a headspace equilibrium procedure (30 min), the SPME needle was inserted into the vial, and the fiber was exposed to the *T. minuta* L. essential oil headspace for 2 min at 21 °C. After sampling, the fiber was thermally desorbed in the glass SPME linear of the GC injection port during 2 min at 280 °C. Split injection (split ratio 1:20) was performed with a SPME Combipal autosampler (CTC Analytics AG, Industriestrasse 20, 4222 Zwingen, Switzerland) on an 6890N chromatograph (Agilent, Massy, France) integrated in a GC×GC-MS-TOF LECO Pegasus 4D instrument (LECO Corporation, St. Joseph, MI, USA) equipped with a cryogenic modulator (LECO Quad Jet Modulator). The two separative columns and the modulator were placed in the oven of the 6890N chromatograph. The first dimension chromatographic separation (column 1) was performed on a SPB-5 capillary column (5% diphenyl-, 95% dimethylpolysiloxane, 30 m length, 0.32 m internal diameterm, 1 μm film thickness; Supelco, St-Germain-en-Laye, France). The second dimension chromatographic separation (column 2) was performed on a DB-17 capillary column (50% dimethyl-, 50% diphenylpolysiloxane, 2.50 m length, 0.178 mm, internal diameter, 0.30 μm film thickness; J&W, Folsom, USA). The column 1 oven was held at 40 °C for 5 min, then ramped up at 3 °C/min to 230 °C, and held for 10 min. The column 2 oven

Table 1. Odorant Compounds Identified in *T. minuta* EO by Direct Injection/AEDA

peak no.[a]	*LRI*[b] HP-1/HP-20M	compound[c]	odor	*T. minuta* L. EO %[d] ± SD [e] (ppm)[f]	*FD*	identification[g]
1	677/867	butenone[N]	buttery, burnt	tr (<1)	1	*LRI*, Odor, MS, Std
	677/-	butanone[N]		tr (<1)		*LRI*, Odor, MS, Std
3	758/1022	ethyl 2-methylpropanoate[N]	fruity, strawberry	0.1 (<1)	9	*LRI*, Odor, MS, Std
4	771/1038	methyl 2-methylbutanoate[N]	fruity	tr (<1)	1	*LRI*, Odor, MS, Std
5	786/1101	hexanal	green, freshly cut grass	tr (<1)	1	*LRI*, Odor, MS, Std
6	806/-	3-methylbut-2-en-1-thiol[t, N]	cabbage, beer, sulfury	-	9	*LRI*, Odor, Std
7	829/1060	ethyl 2-methylbutanoate	fruity	1.0 (<1)	1	*LRI*, Odor, MS, Std
8	833/1069	ethyl 3-methylbutanoate[N]	fruity, pineapple, strawberry	tr (<1)	9	*LRI*, Odor, MS, Std
9	845/-	2-methylfuran-3-thiol[t, N]	nutty	-	9	*LRI*, Odor, Std
12	957/-	oct-1-en-3-one [t, N]	mushroom, metallic	tr (<1)	1	*LRI*, Odor, Std
13	982/1262	octanal	dusty, aldehyde	0.4 (1480 ± 43)	1	*LRI*, Odor, MS, Std
14	984/1146	myrcene	geranium, plastic, citrus	0.1 (1249 ± 20)	1	*LRI*, Odor, MS, Std
16	1069/-	unkown	spicy, curry	-	9	-
18	1083/-	non-1-en-3-ol[t, N]	mushroom[t]	- (<1)	1	*LRI*, Odor
19	1087/1515	linalool	tea, perfume	0.2 (545 ± 40)	3	*LRI*, Odor, MS, Std
21	1123/1363	alloocimene	citrusy	4.5 ± 0.2 (12690 ± 636)	1	*LRI*, Odor, MS, Std
22	1126/1477	(*E*)-tagetone[t]	geranium, bitter, green[t]	0.6 ± 0.3 (10593 ± 348)	1	*LRI*, Odor, MS
24	1135/1531	(*Z*)-tagetone[t]	citrus fruit, bitter, citrus peel[t]	5.1 ± 0.1 (91637 ± 3294)	1	*LRI*, Odor, MS
26	1166/1553	terpinen-4-ol	green, fresh	0.1 (213 ± 7)	3	*LRI*, Odor, MS, Std
27	1168/-	unknown	green, bitter, plastic	-	9	-
29	1189/1498	decanal	nutty, citrusy	0.5 (1579 ± 174)	3	*LRI*, Odor, MS, Std
31	1196/1448	octyl acetate [N]	rotten, floral	0.1 (-)	1	*LRI*, Odor, MS, Std
32	1212/1645	(*E*)-ocimenone [t]	citrusy, perfume[t]	6.9 ± 0.1 (27210 ± 882)	9	*LRI*, Odor, MS
33	1216/1663	(*Z*)- ocimenone [t]	minty, fresh[t]	8.7 ± 0.1 (60338 ± 2028)	1	*LRI*, Odor, MS

[a] Peak number in **Figure 2**. [b] Retention indices on HP-1 and HP-20 M column, determined by injecting a series of *n*-alkanes. [c] Compounds are listed in their elution order on an HP-1 column. [d] Percentage of the total GC/FID area. [e] Quantity in ppm determined by internal standard addition. [f] SD = standard deviation. [g] Method of identification: *LRI* = linear retention index; Odor = comparison of the odorant description of the analyte with the literature and/or the reference compound if injected; MS = mass spectrum; Std = injection of the reference compound. [N]Newly identified in *Tagetes*. [t]Tentatively identified without reference compound injection or no detection by MS.

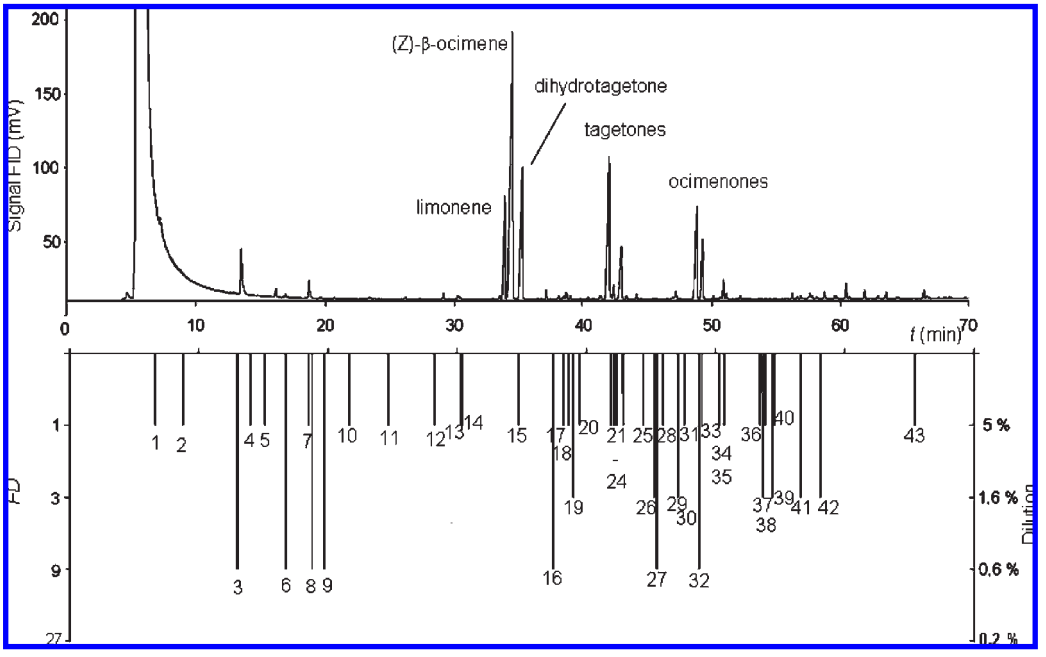

Figure 2. GC/FID chromatogram (above) and AEDA results (below) for *T. minuta* EO. *d* = 3. **1**, butenone/butanone; **3**, ethyl 2-methylpropanoate; **4**, methyl 2-methylbutanoate; **5**, hexanal; **6**, 3-methylbut-2-en-1-thiol[t]; **7**, ethyl 2-methylbutanoate; **8**, ethyl 3-methylbutanoate; **9**, 2-methylfuran-3-thiol[t]; **12**, oct-1-en-3-one[t]; **13**, octanal; **14**, myrcene; **18**, non-1-en-3-ol[t]; **19**, linalool; **21**, alloocimene; **22**, (*E*)-tagetone[t]; **24**, (*Z*)-tagetone[t]; **26**, terpinen-4-ol; **29**, decanal; **31**, octyl acetate; **32**, (*E*)-ocimenone[t]; **33**, (*Z*)-ocimenone[t].

was constantly set at 15 °C higher than the column 1 oven. Ultra high purity helium (Air Liquide U quality, 99.9995%; flow, 1 mL/min) was used as carrier flow. The transfer line was heated at 250 °C, and the ion source set point was 200 °C. The detector voltage was 1600 V. The modulator sequences were modulation period 7 s and hot pulse 0.8 s. Mass spectra (EI) were collected from *m*/*z* 33 to 230 u at a scan rate of 200 spectra/s. Chromatograms were processed using the automated data processing software ChromTOF with a signal-to-noise ratio of 50 (meaning that for the software to recognize a signal as a peak, the S/N-ratio had to be at least 50). The NIST/EPA/NIH mass spectral library (NIST05) (NIST, Gaithersburg, MD, USA) was used for peak identification.

RESULTS AND DISCUSSION

AEDA by direct injection was used in order to obtain information on the relative odor impact of the constituents in the essential

oil itself. Forty-three odorant zones were detected out of which twenty-two could be identified (**Table** 1). The results stated that the EO's overall odor was, despite the presence of largely major compounds such as (*Z*)-β-ocimene (~30% of the total GC/FID area), (*Z*)-ocimenone (~11−12%), dihydrotagetone (25−28%), (*E*)-ocimenone (3−4%), (*Z*)-tagetone (~5−8%), alloocimene (~2−4%), and limonene (~6%), mostly influenced by minor compounds (**Figure 2**). Highest *FD* values were obtained for ethyl 2-methylpropanoate (0.1% of the total GC/FID area), ethyl 3-methylbutanoate (traces), (*E*)-ocimenone (6.9% of the total GC FID area and 2.3% by internal calibration), two tentatively identified thiols (traces), and two yet unknown compounds. Ethylic esters with fruity notes were among the most influential components: ethyl 2-methylpropanoate (fruity, strawberry, *FD* = 9, 0.1%) and ethyl 3-methylbutanoate (fruity, pineapple, strawberry, *FD* = 9, tr). Furthermore, esters contribute to the overall odorant profile, but their influence was minor: methyl 2-methylbutanoate (fruity, *FD* = 1) and ethyl 2-methylbutanoate (fruity, *FD* = 1).

An internal calibration was done in order to give more precise values than the percentage of the total FID area, and the results are also displayed in **Table** 1. The internal calibration results confirm the main compounds. Among the odorant compounds, (*Z*)-tagetone is present in the highest amounts (9.2%), followed by (*Z*)-ocimenone (6.0%) and (*E*)-ocimenone (2.7%). However, the two tagetone isomers (*E*/*Z*) and (*Z*)-ocimenone have low *FD* factors (*FD* = 1), and (*E*)-ocimenone is the only major compound showing an important *FD* value (*FD* = 9), hence considerably influencing the EO's odor (citrusy note).

Two thiols with *FD* = 9 were tentatively identified. Their odorant stimuli could not be identified to any signal in the FID chromatogram. Database research indicated 3-methylbut-2-en-1-thiol at an $LRI_{HP\text{-}1}$ = 806 with a sulfury, cabbage, and beer-like odor, and 2-methylfuran-3-thiol at an $LRI_{HP\text{-}1}$ = 845 with a nutty note. These two compounds were confirmed by injecting the reference compounds in GC-O, but we did not succeed in detecting them by MS. These compounds are thus only tentatively identified, and since this is the first time they are reported in *Tagetes* oil, further enrichment steps will be necessary in order to confirm their identification.

The VIDEO-Sniff method was used in order to detect a maximum number of odorant volatiles due to its detection frequency character with the use of a panel, but also to obtain information on the intensity of the odorant peaks. The use of the 8W-GC-O system allowed us to obtain the response of eight judges (for retention times comprised between 5 and 55 min) in two runs. D-HS was used as a sampling method because it imitates the actual conditions of the odorant perception of the oil by nasal inhalation at body temperature (sample and trap were kept at 40 °C) and is the most suitable method for the concentration of volatile compounds. Theoretically, since the flux is split into eight sniffer ports in 8W-GC-O, a powerful enriching sample preparation step is required. This is not actually necessary for an EO, but since the usual matrices analyzed on the given system are foodstuffs, the 8W-GC-O standard configuration implies D-HS. The D-HS conditions were optimized according to the essential oil's odorant potency and in order to minimize the adsorption of the major monoterpenoids and hence to avoid sniffer saturation (small volume of extract: 1 μL and short extraction time: 7.5 min). According to the VIDEO-Sniff method (8W-GC-O), the eight sniffers found a total amount of 42 distinct odorant zones during the two sniffing runs (5−40 min and 35−55 min). Thirty-seven odorant zones could be identified (**Table** 3). **Figure 3** shows the occurrence of olfactory zones perceived by more than one sniffer or specifically by individual ones and the total olfactory signal (TOS). The occurrence of olfactory zones perceived by only a few sniffers confirm the utility of a panel, e.g., a number of sniffers large enough to limit the risk that certain odorants may not be detected (*3*).

In order to simplify data processing, the vocabulary used to describe the odors is a precious tool. For this purpose, a set of nine olfactory classes was created, and the odor descriptors used by the sniffers were attributed to these classes (**Table** 2). Eight precise classes were generated: balsamic-spicy-pharmaceutical, empyreumatic, floral, fruity, cheesy-lactic, plastic-chemical-solvent, earthy-undergrowth, and green-vegetable. A ninth class called not classified included all odors that could not be described by the sniffers or could not be attributed to one of the classes above. The deconvolution of the TOS according to these different classes gives the mean olfactory signal by classes ($OSC_{Int \times Det}$). This facilitates the interpretation of the results by attributing a color to each class, hence easing the determination of peaks described by an important number of sniffers with vocabulary belonging to the same odor class (**Figure 4**).

Table 2. Distribution of Vocabulary Items Used by the Eight Sniffers in the Nine Olfactory Classes Defined

olfactory class (class color)	vocabulary items
balsamic-spicy-pharmaceutical (mint)	anise, balsamic, tea, fragrant, camphor, chewing gum chlorophyll, chewing gum mint, toothpaste, deodorant, herbs, thyme, hospital, ether, pharmaceutical, medicine, mint, minty, woody, pomade, rosemary, tree bark
empyreumatic (black)	almond, peanuts, roasted peanuts, roasted coffee, chocolate, almond shell, spicy, grilled, roasted, gratin, empyreumatic, bacon, soft bread, walnut, burnt bread, hot bread, bakery, nuts, phenolic, cyclopentanone, grilled sardines
floral (pink)	poppy, poppy seeds, floral, floral-alcoholic, jasmine, rose, shower gel, hygienic products, lavender, perfume, orange blossom, soap, shampoo, linden, herb tea, violet
cheesy-lactic (yellow)	buttery, butter, cream, melted butter, beer, yeast, fried, fat, wax, cheese, rotten Lamb grease, grease, lactone, old lipstick, egg, fat, dust, aldehyde-dusty, milk products, rancid, nonanal, moldy, St. Nectaire cheese, vomit, rotten vomit
fruity (red)	citrus fruit, citrus peel, limonene, marigold, chlorine, pineapple, banana, fruity, fruity-glue, candy, chewing gum, citrus, lemongrass, orange, orange peel, strawberry, fruit candy, fruity-floral, green apple, artificial strawberry, passion fruit, red fruit, lemonade, fruity perfume, orange-citrusy, bitter orange, bitter orange peel, solvent-candy, solvent-fruity
plastic-chemical-solvent (light gray)	bakelite, burnt plastic, chemical, solvent, chlorine, glue, dissolvent, ether, rubber, plastic, hot plastic, insecticide, chemical products, superglue, vegetable-plastic, latex, resinous, vegetable-resinous, pine, alcohol, acetic, detergent, wine
earthy-undergrowth (brown)	wood, pencil wood, tree bark, moss, mushroom, mushroom-sulfury, toadstool, mushroom, sawdust, pencil, herbaceous-earthy, earthy, fungus, mold, earthy, sap, undergrowth, earth, earthy-green
green-vegetable (green)	vegetable, green, genet, geranium, herbaceous, aromatic herbs, crushed herbs, freshly cut grass, olive oil, crude vegetable, green beans, foodstuff, crude green onion, plants, green wood, cassis, peppery, potato, pumpkin, salad, crude cauliflower, sap, sulfury, vegetable-spicy, vegetable-floral, undefined vegetable, dry vegetable
not classified (white)	not identified, pleasant, fruity-floral-salad-vinegar, not describable, cooked meal, cooked fish, sardines, too much noise, vegetable-citrus fruit, animal-cheesy, straw, feet-vegetable, vegetable-balsamic

Table 3. List of Potent Odorants Identified in *T. minuta* Essential Oil by D-HS-8W-GC-O and GC-O-MS

	LRI[b]	*LRI*[b]				detection frequency[f]		
no.[a]	RTX-5	HP-20M	compound[c]	odor description[d]	mean intensity[e]	over 8 judges	%	identification[g]
1	568		2-methylpropanal[N]	roasted, chocolate	2.3	3	37.5	*LRI*, Odor, MS, Std
2	590	867	butenone[N]	buttery	2.6	5	62.5	*LRI*, Odor, MS, Std
			butanone[N, TOF]					*LRI*, Odor, MS, Std
3	651		3-methylbutanal[N]	chocolate	3.0	1	12.5	*LRI*, Odor, MS, Std
4	661		2-methylbutanal[N]	ether	1.0	1	12.5	*LRI*, Odor, MS, Std
6	758	1022	ethyl 2-methylpropanoate[N]	fruity, red fruit	3.1	8	100.0	*LRI*, Odor, MS, Std
7	777	1038	methyl 2-methylbutanoate[N]	fruity, floral, glue, plastic, solvent	3.3	8	100.0	*LRI*, Odor, MS, Std
8	800	1101	hexanal	cut grass, fat, fruity	2.4	5	62.5	*LRI*, Odor, MS, Std
10	844	1042	methyl 3-methylbut-2-enoate[N, TOF]	vegetable, sap, geranium, pumpkin	2.6	5	62.5	*LRI*, Odor, MS, Std
11	850	1060	ethyl 2-methylbutanoate	fruity, pineapple, strawberry	3.8	8	100.0	*LRI*, Odor, MS, Std
12	859	1068	ethyl 3-methylbutanoate[N]	red fruit, strawberry, floral	3.5	2	25.0	*LRI*, Odor, MS, Std
13	876		3-methylbutyl acetate	fruity, banana, solvent	3.3	4	50.0	*LRI*, Odor, MS, Std
14	878	1103	2-methylbutyl acetate	fruity, floral, banana, plastic, glue, solvent	3.3	7	87.5	*LRI*, Odor, MS, Std
15	900		heptanal	phenolic, grilled, herbaceous, almond	3.0	3	37.5	*LRI*, Odor, MS, Std
16	947	1047	α-pinene[TOF]	vegetable, green, camphor	2.0	2	25.0	*LRI*, Odor, MS, Std
17	953		2-methylpropyl butanoate[N, TOF]	fruity	4.6	5	62.5	*LRI*, Odor, MS, Std
18	991		oct-1-en-3-one[t, N]	mushroom	2.9	8	100.0	*LRI*, Odor, Std
19	995		oct-1-en-3-ol[t, N]	mushroom	3.0	7	87.5	*LRI*, Odor, Std
20	1001	1146	myrcene	fruity, green, sardines, geranium, plastic	3.0	5	62.5	*LRI*, Odor, MS, Std
21	1010	1288	(*Z*)-hex-3-enyl acetate	crude vegetable, fruity, citrus fruit	3.8	8	100.0	*LRI*, Odor, MS, Std
22	1021	1144	α-phellandrene	aldehyde, dusty, old lamb fat	3.4	5	62.5	*LRI*, Odor[t], MS, Std
23	1050	1179	limonene	citrus fruit, minty, fruity, floral	3.3	8	100.0	*LRI*, Odor, MS, Std
		1222	(*Z*)-β-ocimene					*LRI*, Odor, MS, Std
24	1065		non-1-en-3-one[t, N]	mushroom[t]	2.3	6	75.0	*LRI*, Odor
25	1070	1287	dihydrotagetone[t]	floral-fruity, tagete, citrus fruit, chemical[t]	3.9	8	100.0	*LRI*, Odor, MS
26	1082		pentyl 3-methylbutanoate[t, TOF]	fruity[t]	1.0	2	25.0	*LRI*, Odor, MS
27	1092		non-1-en-3-ol[t, N]	mushroom, moss[t]	2.4	5	62.5	*LRI*, Odor
28	1098		unknown	beer, yeast, roasted coffee	2.3	4	50.0	
29	1108	1464	camphor[TOF]	vegetable, perfume, floral, plastic	3.0	8	100.0	*LRI*, Odor[t], MS, Std
30	1114		unknown	jasmin, perfume, floral, fruity	2.8	5	62.5	
31	1126	−1363	unknown	hot plastic, floral, fruity, violet	2.3	6	75.0	
32	1142	1363	alloocimene	citrus peel, citrus fruit, grass	3.5	6	75.0	*LRI*, Odor, MS, Std
33	1150	1477	(*E*)-tagetone[t]	deodorant, floral[t]	2.0	2	25.0	*LRI*, Odor, MS
34	1160	1531	(*Z*)-tagetone[t]	minty, plastic, vegetable, geranium, woody[t]	3.2	6	75.0	*LRI*, Odor, MS
35	1165		unknown	fruity, floral, vegetable	4.0	7	87.5	
36	1181		borneol[TOF]	chemical, solvent, woody, moss, sap	3.0	4	50.0	*LRI*, Odor, MS, Std
37	1187	1553	terpinen-4-ol	solvent, vegetable, spicy, earthy, green	3.5	8/16	50.0	*LRI*, Odor, MS, Std
38	1200		decanal	menthol, fruity, green, floral	2.4	5/16	31.3	*LRI*, Odor, MS, Std
39	1237	1645	(*E*)-ocimenone[t]	fruity, floral, citrus fruit[t]	2.5	6	75.0	*LRI*, Odor, MS
40	1237	1663	(*Z*)-ocimenone[t]	minty, chewing-gum[t]	2.7	6	75.0	*LRI*, Odor, MS
41	1243	1723	carvone	fruity, floral, citrus peel, linden	2.4	9/16	56.3	*LRI*, Odor[t], MS, Std
42	1257		unknown	woody, roasted, chocolate, plastic	2.1	11/16	68.8	

[a] Peak number according to **Figure 4**. Detection frequency inferior to 50% is generally considered as noise (*39*) except for compounds easily identified by *LRI*, MS, and odor. [b] *LRI* on apolar (RTX-5 of the 8W-GC-O device) and polar column (HP-20M) determined with an series of *n*-alkanes. [c] Compounds are listed in elution order on an RTX-5 column. [d] Main odor descriptors given by the judges. [e] Mean intensity given by the eight judges (on a scale from 1 to 5). [f] Detection frequency over eight judges. [g] Identification method: *LRI* = linear retention index, odor = comparison of odor descriptors to our homemade GC-O database ; MS = mass spectrum ; Std = reference compound injection. [N]Compounds newly identified in *Tagetes minuta*. [TOF]Mass spectra obtained by GC×GC-TOFMS. [t]Tentatively identified = reference compounds not injected or not detection by MS. Odor[t] = odor descriptor of reference compound not exactly matching the target compound.

By taking into account the sniffers' vocabulary, we are able to distinguish compounds belonging to the different olfactory classes. Twelve fruity signals were found of which 10 are positively identified. Among these are eight esters (**Table** 3). The most influent ones are ethyl 2-methylpropanoate that was detected by 100% of the sniffers and attributed to the fruity class with 88% consensual description (peak no. 6, red fruit odor), and methyl 2-methylbutanoate that was detected by all sniffers, but its odor descriptors were divided into several classes (peak no. 7, fruity, plastic). Ethyl 2-methylbutanoate is also detected by 100% of the panel and attributed to the fruity class with 100% consensus (peak no. 11, fruity, pineapple). This signal is directly followed by peak no. 12, ethyl 3-methylbutanoate (red fruit), which is detected by only 25% of the panel, but with a mean intensity of 3.5 (*37*, *38*). It is likely that some judges were incapable of differentiating between those two esters eluting closely with fruity notes. The fact that fruity notes play an important role in the essential oil's odor is in agreement with the strong fruity note in its overall odor and confirms the results obtained by AEDA.

Peak no. 2 was detected by five judges (63% of the panel) and described as buttery and could be attributed to the cheesy-lactic class with an 80% consensus. In absence of diacetyl (butane-2,3-dione, absence confirmed by GC×GC-TOFMS), the odor was attributed to butenone but also to butanone, as found by 2DGC and confirmed by standard injection.

Peak no. 18 was detected by all sniffers and described as mushroom (earthy-undergrowth) with 75% consensus. It shows an important $OSC_{Int \times Det}$ value, but the compound eluting in important amounts at the retention time was sabinene, which does not smell like mushrooms. The GC-O data bank search drew our attention to oct-1-en-3-one, known for its mushroom note. Its

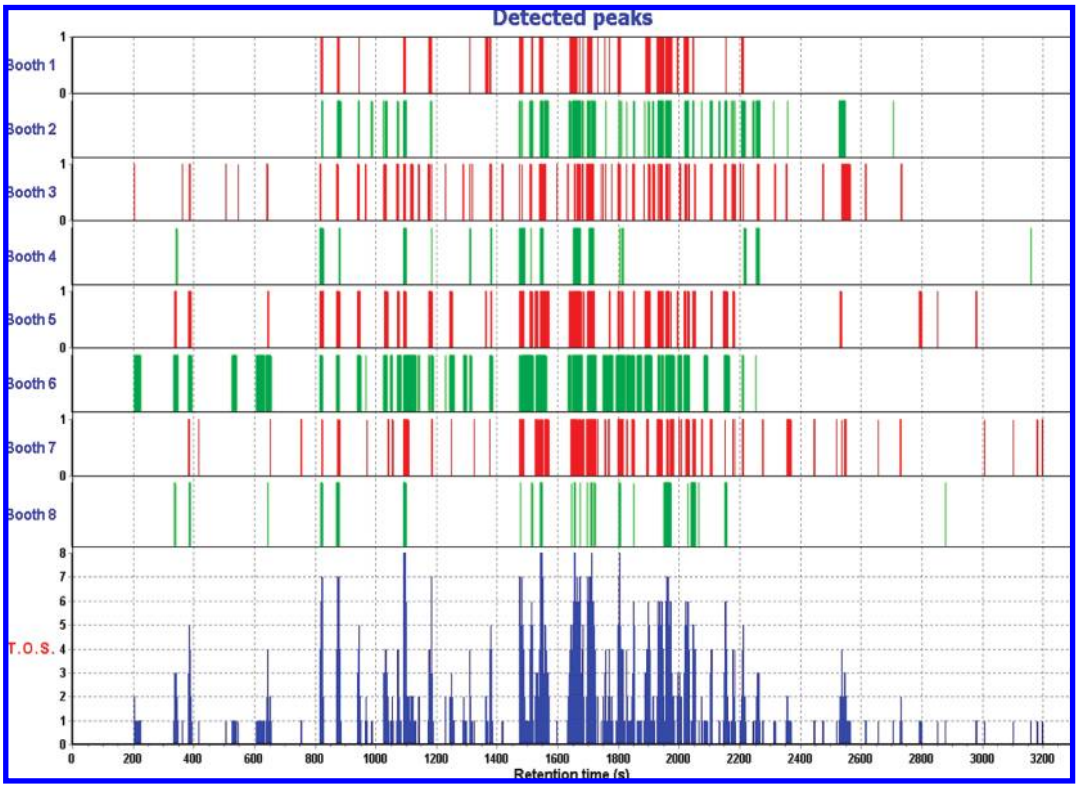

Figure 3. Raw data from individual detection of the eight sniffers (booths 1−8) and total olfactory signal (TOS) expressed in number of detections.

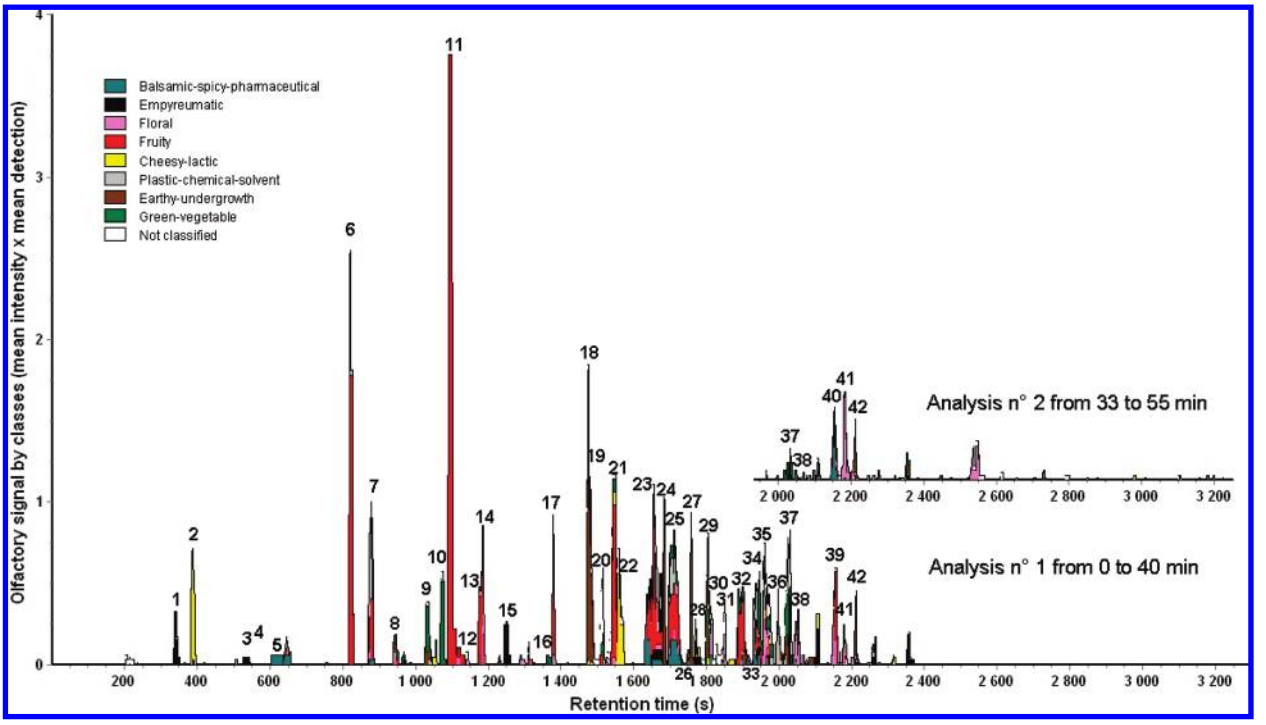

Figure 4. Mean olfactory signal by classes ($OSC_{Int \times Det}$). The breakdown of the mean total olfactory signal ($TOS_{Int \times Det}$) into nine classes shows the odorant zones belonging to a given olfactory class. Labels corresponding to the peak numbers are given in **Table 3**.

odor was confirmed by injection of the reference compound in GC-O. However, apparently this compound is present in too low of quantity to be detected by MS and has hence been denoted as tentatively identified. Further enrichment steps will have to be done in order to render MS detection possible.

When compared to direct-injection/AEDA, D-HS-8W-GC-O/VIDEO-Sniff allowed the identification of 20 additional compounds. This might be due to the enrichment in volatile components achieved by D-HS and to the use of a panel of sniffers. Indeed, several aldehydes with chocolate and ether notes were identified by 8W-GC-O: 2-methylpropanal was perceived by only three panelists and attributed to the empyreumatic class with 100% consensus. 3-Methylbutanal and 2-methylbutanal were detected and described by only one sniffer, but odor descriptions

Table 4. Compounds Newly Identified in *T. minuta* EO

compound	identification[a]
2-methylpropanal^{GC-O}	*LRI*, Odor, MS, Std
butenone	*LRI*, Odor, MS, Std
butanone$^{TOF,GC-O}$	*LRI*, Odor, MS, Std
3-methylbutanal^{GC-O}	*LRI*, Odor, MS, Std
2-methylbutanal	*LRI*, Odor, MS, Std
ethyl 2-methylpropanoate	*LRI*, Odor, MS, Std
methyl 2-methylbutanoate	*LRI*, Odor, MS, Std
3-methylbut-2-en-1-thiol $^{t,GC-O}$	*LRI*, Odor, Std
methyl 3-methylbut-2-enoate $^{TOF, GC-O}$	*LRI*, Odor, MS, Std
ethyl 3-methylbutanoate	*LRI*, Odor, MS, Std
2-methylfuran-3-thiol$^{t, GC-O}$	*LRI*, Odor, Std
oct-1-en-3-one$^{t, GC-O}$	*LRI*, Odor, Std
oct-1-en-3-ol$^{t, GC-O}$	*LRI*, Odor, Std
non-1-en-3-one$^{t, GC-O}$	*LRI*, Odor
pentyl 3-methylpentanoate$^{t, TOF, GC-O}$	*LRI*, Odor, MS
non-1-en-3-ol$^{t, GC-O}$	*LRI*, Odor
octyl acetate	*LRI*, Odor, MS, Std

[a] Identification method: *LRI* = linear retention index, odor = comparison of odor descriptors to our homemade GC-O database ; MS = mass spectrum ; Std = reference compound injection. ^{TOF}Mass spectra obtained by GC×GC-TOFMS. ^{t}Tentatively identified = reference compounds not injected or not detection by MS. $^{GC-O}$Compound detected thanks to GC-O analyses (AEDA or VIDEO-Sniff).

and mass spectra led to a positive identification. We also found several esters with fruity notes such as 2- and 3-methylbutyl acetates (fruity, banana notes) and a pentyl ester. Oct-1-en-3-one, a trace compound in the EO and detected by AEDA with a *FD* value of 1, gives a high $OSC_{Int \times Det}$ in VIDEO-Sniff since it is detected by all eight sniffers with a mean intensity of 2.8 on a scale from one to five.

Both methods allow one to analyze odorant compounds with apolar retention indices up to 1400 with the used sampling technique and only one compound elutes at $LRI_{HP\text{-}1} = 1550$ in AEDA. The loss of information by using D-HS is negligible.

Five compounds were identified only by direct-injection/AEDA and not by D-HS-8W-GC-O/VIDEO-Sniff: octanal ($FD = 1$), linalool ($FD = 3$), and octyl acetate ($FD = 1$) were confirmed by injection of the reference compounds in GC-MS and GC-O. Two thiols were tentatively and for the first time identified in *Tagetes minuta* with a $FD = 9$: 3-methylbut-2-en-1-thiol and 2-methylfuran-3-thiol. Since we were not able to detect these compounds by MS, their identification has to be considered as tentative.

It can be concluded that both methods are in agreement since both bring out the influence of numerous esters (fruity notes) on the essential oil's odor with ethyl 2-methylpropanoate and ethyl 2/3-methylbutanoate as the most influential compounds. Each method has led to compounds not detected with the other one, which is probably due to the sample preparation method. Some of the major compounds (limonene and (*Z*)-β-ocimene, dihydrotagetone) were not detected at the given concentrations by AEDA since a choice had to be made in order to work at a starting concentration that did not cause sniffer saturation. The use of a panel in the VIDEO-Sniff method allowed the identification of several additional aldehydes, esters, and trace compounds.

The combined use of AEDA by direct injection and D-HS-VIDEO-Sniff in 8W-mode gives good knowledge of the odor impact compounds present in the essential oil. All in all, our analyses identified 17 new compounds that have never been identified in *Tagetes* before (denoted as ^{N} in the tables and listed separately in **Table** 4, designated as new according to the literature on *Tagetes* constituents); some have yet to be confirmed. Eleven of these 17 compounds were found thanks to GC-O analyses. Globally, 2DGC-MS contributed by rendering the identification of seven odor impact compounds (five of them with detection frequencies ≥50%) possible that were not detected by 1DGC. Three of these seven compounds are newly found in *Tagetes*.

Concerning the practical aspects, 8W-GC-O/VIDEO-Sniff considerably reduced analysis time. After optimization (which is necessary for both methods), eight individual aromagrams of eight different judges were obtained in 55 min by 8W-GC-O/VIDEO-Sniff. AEDA took four dilutions for no odor to be perceived anymore and thus four times a 60 min chromatography run. Since the run had to be split up in order to avoid sniffer fatigue, this makes eight analyses of about 30 min in order to obtain AEDA results for one sniffer. Taking into account that the analyses have to be conducted twice to ensure repeatability, AEDA with one panelist takes 480 min in order to obtain a reliable result, which is 8 h of GC-O. Even if AEDA gave satisfying results with only two judges and both methods pointed out the same components as main odorants, 8W-GC-O/VIDEO-Sniff allowed the identification of more compounds in a considerably shorter time (about eight times shorter) with more reliable results thanks to the sniffer panel. AEDA should hence be considered as a screening method which can be done with few panelists. As for analysis time, multiport systems are the more convenient and efficient choice, but those systems are still quite rare.

The strength of detection frequency, intensity, and hybrid methods lies mainly in the use of a panel, but VIDEO-Sniff presents the advantage of including sniffer vocabulary and eases visual interpretation of the results.

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