Research Article

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Comparison of the characterisation of the fruit-like aroma of *Teucrium flavum* L. subsp *flavum* by hydrodistillation and solid-phase micro-extraction

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Abstract

BACKGROUND: *Teucrium* species (Lamiaceae) are interesting for the food industry since many of them are used in the preparation of flavoured wines and beers, herbal teas, bitters and liqueurs. On this basis, the analysis of the aroma components of *Teucrium flavum* L. subsp. *flavum* growing in central Italy was carried out by means of both hydrodistillation (HD) and headspace solid-phase micro-extraction (HS-SPME), coupled with GC/FID and GC/MS.

RESULTS: A total of 102 components were identified in the essential oils, representing 99.0-99.3% of the total oils. Sesquiterpenes hydrocarbons constituted the major fraction (48.5–49.4%), with the apple-like flavour (*Z*,*E*)-α-farnesene being the major component. HS-SPME allowed the analysis of the volatiles not only emitted by specific plant parts, but also by different parts within a single flower: flower calyx afforded the highest contribution, in terms of volatiles, to the aroma of the plant.

CONCLUSION: The chemical profile of the volatile fraction obtained by HD and HS-SPME, demonstrated the plant fruit-like aroma, confirming the usefulness for flavouring wines, bitters and other kind of beverages, and also suggested other applications, as aroma and taste enhancer in food processing. In particular, SPME resulted in a very useful technique, which permits a choice between the part of the plant which has the highest concentration of a specific fragrance, and therefore establishes the best way of sampling during industrial applications of aromatic plants.

Keywords: *Teucrium flavum* subsp. *flavum*; Lamiaceae; essential oil; SPME; GC/FID; GC/MS

INTRODUCTION

Teucrium flavum L., belonging to the Lamiacae family, Sect. *Chamaedrys* (Miller) Schreber, is an evergreen, branchy, semi-woody shrub, up to 60 cm tall, distributed in the Mediterranean Basin on rocky places.[1] It occurs in Italy with two subspecies: the subsp. *flavum* L. distributed along the peninsula and in the islands; the subsp. *glaucum* (Jord. & Fourr.) L. growing only in Basilicata, Sicily and Sardinia.[2] In Italian folk medicine the infusion of the top flowers of this plant was used as antipyretic and antiseptic, whilst the decoction of the leaves was applied directly to the skin as a cicatrizant.[3]

Previous phytochemical and pharmacological studies showed that this plant contained norclerodane diterpenoids, namely teuflin and teuflidin,[4,5] and that its extracts exert anti-inflammatory,[6] analgesic,[7] hypotensive[8] and antioxidant[9] activity.

Like many Lamiaceae, the species of the genus *Teucrium* contain volatile compounds that contribute to the particular fragrance of these plants, which are used in the preparation of flavoured wines, bitters and liqueurs, or as a hop substitute for flavouring beer.[10]

To the best of our knowledge, there are very few other studies investigating the essential oil of *T. flavum*: one from Iran,[11] one from Serbia and Montenegro,[12] two from Greece,[13,14] and one from Italy.[15] Only in the latter study was the subspecies *flavum* investigated; researchers found as major volatiles *trans*-4-methoxycinnamic acid and borneol in leaves, borneol and α-pinene in bracts, β-cubebene and α-farnesene in calyces, and *trans*-4-methoxycinnamic acid in corollas. However, it is necessary to clarify that, in this study, determined compounds were extracted with chloroform from plant material, and consequently some high-boiling point volatiles were also obtained, as aromatic acid derivatives, which cannot, in the strict sense, be considered essential oil components. From the studies cited above, it is seen

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that volatiles from this plant are dominated by sesquiterpenes, with (*E*)-caryophyllene and germacrene D as the major components in samples from Iran and Greece,[11,13] caryophyllene, along with 4-vinyl guaiacol, in the second study conducted on Greek samples,[14] and β-bisabolene being the major component in the sample from Serbia and Montenegro.[12]

In this study we carried out a characterisation of volatile fraction of *T. flavum* subsp. *flavum* growing in central Italy by using both hydrodistillation (HD) and headspace solid-phase micro-extraction (SPME), in combination with gas chromatography (GC) and gas chromatography coupled to mass spectrometry (GC/MS).

To the best of our knowledge this is the first report about study of the fruit-like aroma of *Teucrium flavum* L. subsp *flavum* performed by the SPME technique. Besides extraction of essential oils by means of classical HD, in this study we used the SPME technique in order to characterise volatiles from different plant parts, even from different parts of a single flower. In fact, the study of floral scents within a single flower may reveal molecules useful to both the food industry and perfumery.

EXPERIMENTAL

Plant material

Aerial parts of *T. flavum* subsp. *flavum* were collected at flowering time in June 2007 and 2008 in a rocky place sited in Marche (central Italy): name of locality, Bistocco; GPS coordinates, 43°04′ 33″ N, 13°01′ 35″ E. Plant material collected in 2007 was air-dried for 1 week protected from the light, while that collected in 2008 was used as a fresh sample. A voucher specimen was identified by Dr Maggi and deposited in the Herbarium Camerinensis (Department of Environmental Sciences, Section of Botany and Ecology, University of Camerino, Italy), under the accession code CAME 13 412; it is also available at the following website: htpp://erbariitaliani.unipg.it). For SPME analysis, dry and fresh plant materials (flowering aerial parts, leaves, stems and flowers) were ground with a blender MFC model DCFH 48 IKA-WERK (IKA, Staufen, Germany) using filters of 1 and 2 mm size in diameter, respectively. In addition, both dry and fresh whole flowers and different flower parts (corolla and calyx) were separately submitted to SPME extraction.

Hydrodistillation

Hydrodistillation for extraction of essential oils were performed on the aerial parts (fresh and air-dried) with a Clevenger-type apparatus (Ciro Donati, Rome, Italy) for 3 h, using hexane (3 mL) as collector solvent. Oils were dried over anhydrous sodium sulfate, then solvent was evaporated under a N_2 flow. Afterwards, they were stored in sealed vials protected from the light at −20 °C before GC/FID and GC/MS analyses (Agilent, Santa Clara CA, USA). Three essential oils were obtained by HD from three different samples coming from the collection site. They were subsequently analysed by GC/FID and GC/MS. The oil yield (w/w, 0.02–0.05%) was estimated on a dry weight basis. Hydrodistilled oils had a typical fruit-like smell.

Solid-phase micro-extraction analysis

The silica fibres and the manual SPME holder were purchased from Supelco (Bellefonte, PA, USA). Three fibres were tested and compared: polydimethylsiloxane (PDMS, 100 μm), polydimethylsiloxane–divinylbenzene (PDMS/DVB, 65 μm) and Stableflex divinylbenzene–carboxen–polydimethylsiloxane (DVB/CAR/PDMS, 50/30 μm). To optimise the extraction conditions different factors were considered: extraction temperature, extraction time, particle size, water addition, desorption time, amount of plant material. The coating of all fibres was 1 cm long. Before GC/FID and GC/MS analysis, each fibre was conditioned in the injector of the GC system, according to the instructions provided by the manufacturer.

Dry and fresh plant materials (30 mg of grounded flowering aerial parts, leaves, stems and flowers; single whole flower, calyx and corolla) were hermetically sealed in a 4 mL vial with a polypropylene hole cap and PTFE/silicone septa (Supelco) and equilibrated in a thermostatic bath at the desired temperature. Then, the SPME device was inserted into the sealed vial by manually penetrating the septum and the fibre was exposed to the plant material headspace during the extraction time. Before sampling, single whole flower and its parts were weighed (three determinations) and the average calculated. Experimental conditions were set as follows: extraction temperature, 60 °C; extraction time, 30 min; particle size, 1 mm (dry material) and 2 mm (fresh material); water addition, 60 μL; desorption time, 3 min (at 250 °C in splitless mode). For each part investigated, SPME analysis was conducted in triplicate. After sampling, the SPME device was immediately inserted into the GC injector and the fibre thermally desorbed. No reconditioning was needed for each fibre before next sampling.

Gas chromatography and gas chromatography–mass spectrometry

GC/FID analysis of the volatile components was carried out using an Agilent 4890D (XXXXX, XXXXX) instrument coupled to a flame ionisation detector (FID). Compounds were separated on a HP-5 capillary column (5% phenylmethylpolysiloxane, 25 m, 0.32 mm i.d.; 0.17 μm film thickness) (J & W Scientific, Folsom, CA, USA), with the following temperature program: 5 min at 60 °C, subsequently 4 °C min^{-1} up to 220 °C, then 11 °C min^{-1} up to 280 °C, held for 15 min; injector and detector temperatures, 280 °C (250 °C for SPME); carrier gas, helium (1.4 mL min^{-1}); injection volume of 1 μL, split ratio, 1 : 34. A mixture of aliphatic hydrocarbons (C_8 – C_{30}) (Sigma, Milan, Italy) in hexane, was directly injected into the GC injector or loaded onto the SPME fibre and injected using the above temperature program, in order to calculate the retention indices (as Kovats indices) of each extracted compound by using both HD and HS-SPME. All GC/FID analyses were conducted in triplicate.

GC/MS analysis was performed using an Agilent 6890N gas chromatograph coupled to a 5973N mass spectrometer, equipped with a HP-5MS capillary column (5% phenylmethylpolysiloxane, 30 m, 0.25 mm i.d., 0.1 μm film thickness) (J & W Scientific). The GC was programmed at 60 °C for 5 min, then ramp at 4 °C min^{-1} to 220 °C, then 11 °C min^{-1} up to 280 °C, held for 15 min, finally 11 °C min^{-1} up to 300 °C, held for 5 min; carrier gas: helium; flow rate: 1.0 mL min^{-1}; injector and transfer line temperatures: 280 °C (250 °C for SPME); injection volume: 2 μL; split ratio: 1 : 50; scan time: 75 min; acquisition mass range: 29–400 *m/z*. All mass spectra were acquired in electron-impact (EI) mode with an ionisation voltage of 70 eV.

Identification and quantification of volatile components

The identification of volatile components was based on computer matching with the WILEY275, NIST05, and ADAMS libraries, as well as by comparison of the mass spectra and retention indices (RIs) with those reported in the literature.[16,17] In addition, a

Table 1. Determinations of GC/FID response factor (RF) for different classes of volatiles occurring in *Teucrium flavum* subsp. *flavum*

Grouped compounds	Mean ± SD	RF
Terpenoids		
Monoterpenes hydrocarbons		
β-Pinene	1.04 ± 0.01	1.09 ± 0.06
Limonene	1.15 ± 0.01	
γ-Terpinene	1.04 ± 0.01	
p-Cymene	1.14 ± 0.01	
Oxygenated monoterpenes		
1,8-Cineole	1.29 ± 0.01	1.36 ± 0.09
Linalool	1.33 ± 0.01	
Camphor	1.32 ± 0.01	
Terpinen-4-ol	1.29 ± 0.01	
Verbenone	1.42 ± 0.01	
Nerol	1.53 ± 0.01	
Carvone	1.35 ± 0.01	
Sesquiterpenes hydrocarbons		
(*E*)-Caryophyllene	1.11 ± 0.01	1.11 ± 0.00
α-Humulene	1.11 ± 0.01	
Oxygenated sesquiterpenes		
Caryophyllene oxide	1.20 ± 0.01	1.20 ± 0.00
Aliphatics		
Alcohols		
1-Octen-3-ol	1.30 ± 0.01	1.38 ± 0.11
Dodecanol	1.45 ± 0.01	
Esters		
Isobornyl acetate	1.39 ± 0.01	1.39 ± 0.01
Isobornyl isovalerate	1.38 ± 0.01	
Aldehydes		
Octanal	1.50 ± 0.01	1.49 ± 0.03
Dodecanal	1.47 ± 0.01	
Aromatics		
Benzaldehyde	1.28 ± 0.01	1.32 ± 0.05
p-Vinylanisole	1.36 ± 0.01	

home-made library, based on the analyses of reference oils and commercially available standards, was used. Whenever possible, components were identified also by comparing the retention times of the chromatographic peaks with those of authentic compounds (available in the authors' laboratory) run under the same conditions.

The relative amounts of volatile components, expressed as percentages, were obtained by FID peak-area normalisation, by calculating the response factor (RF) of the FID for eight different classes of volatiles (Table 1). Owing to the large number of identified compounds and the non-availability of commercial standards for many of them, compounds of each of the eight classes were assumed to have the same quantitative GC correction factor. Standard compounds, each representing the determined chemical classes, were selected among those available in the authors' laboratory (listed in Table 1). For a high level of reliability, more than one standard for each class was considered, when possible. Five replicates of mixtures with equal amounts of internal standards (octane and octadecane) and representative compounds were prepared; their final concentrations were in the range 0.35–0.40 mg mL^{-1}, taking into account the purity determined for each of them at GC/FID. A correction factor of 1 was assumed for compounds that did not belong to any of these classes. The response factors (RFs) were means of the response factors (which were themselves the average of five runs) produced by each standard compound within a chemical class. The formula used was $RF = C_{analyte}/[(A_{analyte}/A_{is})] \times C_{is}$, where $C_{analyte}$ is the concentration of the standard representing a chemical group (e.g. β-pinene for monoterpene hydrocarbons), $A_{analyte}$ its absolute peak area, A_{is} is the average of the absolute peak areas of octane and octadecane and C_{is} their concentrations. Using the generalised response factor for compounds within the eight classes, the derived quantitative data may be considered as an approximation of the absolute quantification.

RESULTS AND DISCUSSION

In this study, two techniques were considered for the extraction of the volatile compounds from *T. flavum* subsp. *flavum*: HD and HS-SPME. In particular, HD was performed on flowering aerial parts, whilst HS-SPME was carried out on flowering aerial parts, leaves, stems, flowers, and on a single whole flower and its parts (calyx and corolla).

Essential oil analysis

Composition of the essential oils obtained from dry and fresh aerial parts of *T. flavum* subsp. *flavum* is given in Table 2. One hundred and two components were identified, representing 99.0–99.3% of the oils under study. The oils were dominated by terpenoids (82.7–83.3%), while aliphatics compounds constituted a small fraction (14.4–16.5%). The major components were (*Z*,*E*)-α-farnesene (11.5–14.9%), 11-α-*H*-himachal-4-en-1-β-ol (6.2–10.1%), (*E*)-β-farnesene (5.7–7.3%), β-bisabolene (5.0–7.5%), linalool (7.6–7.8%) and germacrene D (5.5–6.6%). Sesquiterpenes constituted the major fraction of the oil (59.6–61.6%) with hydrocarbons (48.5–49.4%) being the most abundant. These data are in accordance with those reported in literature, confirming that the essential oil of *Teucrium* species is dominated by sesquiterpenes.[10–13,18] Monoterpenes constituted the second fraction (20.9–23.1%) of the oils, with α-pinene (4.5–5.3%), β-pinene (3.1–4.5%) and limonene (3.0–3.5%) as the most abundant among the hydrocarbons and linalool (7.6–7.8%) among the oxygenated compounds. No evident qualitative and quantitative differences were noticed between essential oils obtained from dry and fresh flowering aerial parts. If we compare our results with those occurring in literature concerning the same entity,[15] we notice both qualitative and quantitative differences. In fact, borneol, one of the most abundant component in leaves (27.86%) and bracts (54.07%) of the samples from Elba Island, was completely lacking in our sample, as *trans*-4-methoxycinnamic, whilst α-pinene, that was abundant in bracts (21.09%) and calyces (16.19%) of samples from Elba Island, was present only in small amounts in our samples. Moreover, samples from Elba Island were qualitatively poorer than ours, with only 14 volatiles reported, versus 102 detected in our study. Significant were the differences detected with respect to the essential oils from other countries, in particular with those from Greece,[14] which resulted qualitatively poorer (58 vs. 102 components) and with different major components (caryophyllene and 4-vinyl guaiacol).

If we compare essential oil compositions with SPME extraction of flowering aerial parts (Table 2), we can notice

Table 2. Chemical composition of the essential oils obtained by HD and SPME from dry and fresh flowering aerial parts of *Teucrium flavum* subsp. *flavum*

		HD[a]		SPME[a]		
Component[b]	RI[c]	Dry	Fresh	Dry	Fresh	Identification methods[d]
(2*E*)-Hexenal	857	t	1.4 ± 1.1	0.1 ± 0.0	0.3 ± 0.0	MS, RI
n-Hexanol	872	0.5 ± 0.1	1.1 ± 0.2	0.7 ± 0.1	0.4 ± 0.0	MS, RI
α-Thujene	922	–	t	–	–	MS, RI
α-Pinene	933	5.3 ± 0.4	4.5 ± 1.4	0.6 ± 0.0	0.4 ± 0.1	MS, RI, std
Camphene	949	t	t	–	–	MS, RI, std
Benzaldehyde	953	–	t	0.1 ± 0.0	t	MS, RI, std
Sabinene	969	t	t	–	–	MS, RI, std
β-Pinene	976	4.5 ± 0.5	3.1 ± 1.2	0.5 ± 0.0	0.3 ± 0.1	MS, RI, std
1-Octen-3-ol	984	2.9 ± 0.2	4.8 ± 0.8	1.0 ± 0.0	1.2 ± 0.0	MS, RI, std
Myrcene	993	0.6 ± 0.0	0.5 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	MS, RI, std
3-Octanol	1002	–	0.1 ± 0.1	–	–	MS, RI
(2*E*,4*E*)-Heptadienal	1007	0.1 ± 0.1	t	–	–	MS, RI
p-Cymene	1024	t	t	–	–	MS, RI, std
Limonene	1031	3.5 ± 0.6	3.0 ± 0.9	0.5 ± 0.0	0.3 ± 0.0	MS, RI, std
(*Z*)-β-Ocimene	1045	0.3 ± 0.0	0.4 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	MS, RI
Benzene acetaldehyde	1054	t	t	–	–	MS, RI
(*E*)-β-Ocimene	1056	t	0.1 ± 0.1	t	t	MS, RI
Isopentyl butanoate	1069	t	t			MS, RI
3,5-Octadien-2-one	1084	–	–	t	–	
Terpinolene	1089	t	t	–	–	MS, RI, std
Linalool	1103	7.6 ± 0.9	7.8 ± 1.2	4.0 ± 0.2	3.4 ± 0.0	MS, RI, std
Nonanal	1109	1.1 ± 0.0	1.0 ± 0.1	t	0.2 ± 0.0	MS, RI
n-Amyl isovalerate	1113	0.6 ± 0.0	0.4 ± 0.2	0.4 ± 0.0	t	MS, RI
β-Thujone	1119	0.5 ± 0.4	–	–	–	MS, RI, std
1-Octen-3-yl acetate	1121	0.7 ± 0.1	0.8 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	MS, RI
α-Campholenal	1124	t	–	–	–	MS, RI
3-Octanol, acetate	1134	t	t	1.0 ± 0.1	0.1 ± 0.0	MS, RI
trans-Pinocarveol	1137	t	t	0.2 ± 0.0	–	MS, RI
Isobutyl hexanoate	1149	t	t	–	–	MS, RI
p-Vinylanisole	1160	0.8 ± 0.0	0.5 ± 0.3	–	–	MS, RI, std
Pinocarvone	1167	t	t	0.1 ± 0.0	–	MS, RI
Terpinen-4-ol	1182	t	t			MS, RI, std
Butanoic acid, hexyl ester	1190	–	–	0.1 ± 0.0	0.1 ± 0.0	
α-Terpineol	1194	0.2 ± 0.1	0.1 ± 0.1	–	–	MS, RI, std
Methyl salicylate	1195	t	t	0.3 ± 0.0	0.3 ± 0.0	MS, RI
Hexanoic acid, butyl ester	1198	0.3 ± 0.2	0.1 ± 0.1			MS, RI
Myrtenol	1196	–	–	t	t	
Decanal	1211	t	t	0.2 ± 0.0	0.1 ± 0.0	MS, RI
Verbenone	1205	–	–	0.1 ± 0.0	–	
β-Cyclocitral	1219	t	t	0.1 ± 0.0	–	MS, RI
n-Hexyl 2-methyl butanoate	1244	t	t	0.2 ± 0.0	0.1 ± 0.0	MS, RI
cis-3-Hexenyl isovalerate	1239	–	–	t	0.1 ± 0.0	
Carvone	1244	–	–	t	t	
Hexyl isovalerate	1249	t	t	0.1 ± 0.0	t	MS, RI
Isoamyl hexanoate	1259	1.7 ± 0.0	1.5 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	MS, RI
(2*E*)-Decenal	1268	t	0.1 ± 0.1	t	–	MS, RI
Dihydroedulan I	1286	t	t	–	–	MS, RI
trans-Linalool oxide acetate (pyranoid)	1291	0.6 ± 0.1	1.5 ± 0.1	1.8 ± 0.0	2.0 ± 0.1	MS, RI, std
Theaspirane A	1296	t	t	–	–	MS, RI
Tridecane	1300	–	0.3 ± 0.2	–	–	MS, RI, std
Undecanal	1305	–	–	t	–	
Theaspirane B	1315	t	t	–	–	MS, RI
Isoamyl heptanoate	1319	t	t	0.1 ± 0.0	0.1 ± 0.0	MS, RI
p-Vinyl-guaiacol	1320	0.1 ± 0.1	t	–	–	MS, RI
Methyl geranate	1328	t	t	0.3 ± 0.0	0.3 ± 0.0	MS, RI

Table 2. (*Continued*)

Component[b]	RI[c]	HD[a] Dry	HD[a] Fresh	SPME[a] Dry	SPME[a] Fresh	Identification methods[d]
Hexyl tiglate	1336	t	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	MS, RI
α-Longipinene	1350	–	–	0.2 ± 0.0	0.2 ± 0.0	
Eugenol	1365	t	t	t	t	MS, RI, std
α-Copaene	1373	0.2 ± 0.0	t	0.2 ± 0.0	0.3 ± 0.0	MS, RI, std
Isoledene	1375	–	–	t	0.1 ± 0.0	
3,4-Dimethoxystyrene	1379	0.6 ± 0.0	0.1 ± 0.1	–	–	MS, RI
Geranyl acetate	1379	–	–	0.1 ± 0.0	t	
β-Bourbonene	1384	0.4 ± 0.0	0.3 ± 0.2	1.6 ± 0.3	0.9 ± 0.0	MS, RI
(*E*)-β-Damascenone	1389	0.3 ± 0.0	0.2 ± 0.1	–	–	MS, RI
hexyl hexanoate	1392	t	0.3 ± 0.2	–	–	MS, RI
7-epi-Sesquitujene	1392	–	–	0.7 ± 0.0	0.6 ± 0.0	
Sesquitujene	1405	–	–	0.1 ± 0.0	0.2 ± 0.0	
α-Gurjunene	1407	t	t	–	–	MS, RI, std
(*E*)-Caryophyllene	1414	5.7 ± 0.1	5.1 ± 0.1	5.1 ± 0.0	8.4 ± 0.0	MS, RI, std
β-Copaene	1426	0.1 ± 0.1	0.1 ± 0.1	0.6 ± 0.0	0.8 ± 0.0	MS, RI
trans-α-Bergamotene	1435	0.4 ± 0.0	0.2 ± 0.1	0.7 ± 0.0	0.5 ± 0.0	MS, RI
(*Z*)-β-Farnesene	1443	1.3 ± 0.1	1.6 ± 0.0	2.2 ± 0.0	1.6 ± 0.0	MS, RI
α-Humulene	1450	2.8 ± 0.1	2.5 ± 0.1	t	t	MS, RI, std
allo-Aromadendrene	1456	t	t	1.0 ± 0.0	1.1 ± 0.1	MS, RI, std
(*E*)-β-Farnesene	1460	7.3 ± 0.1	5.7 ± 0.2	12.3 ± 0.1	13.3 ± 0.2	MS, RI
Germacrene D	1478	5.5 ± 0.2	6.6 ± 0.3	3.1 ± 0.0	13.2 ± 0.1	MS, RI, std
γ-Curcumene	1481	t	t	–	–	MS, RI
ar-Curcumene	1484	t	t	–	–	MS, RI
(*E*)-β-Ionone	1488	0.2 ± 0.0	t	0.4 ± 0.0	0.2 ± 0.0	MS, RI
Bicyclogermacrene	1493	0.8 ± 0.0	0.9 ± 0.0	–	–	MS, RI
α-Zingiberene	1495	2.1 ± 0.1	2.6 ± 0.1	6.0 ± 0.0	6.5 ± 0.0	MS, RI, std
(*Z*,*E*)-α-Farnesene	1498	11.5 ± 0.2	14.9 ± 0.5	33.9 ± 0.2	27.3 ± 0.4	MS, RI
β-Bisabolene	1509	7.5 ± 0.3	5.0 ± 0.4	0.5 ± 0.0	0.6 ± 0.0	MS, RI
β-Curcumene	1512	0.3 ± 0.1	0.2 ± 0.1	t	t	MS, RI
δ-Cadinene	1522	t	t	0.3 ± 0.0	0.4 ± 0.0	MS, RI
β-Sesquiphellandrene	1524	1.2 ± 0.0	1.4 ± 0.1	2.8 ± 0.0	2.6 ± 0.0	MS, RI
(*E*)-γ-bisabolene	1529	t	t	t	t	MS, RI
trans-α-Bisabolene	1537	1.3 ± 0.1	1.8 ± 0.1	2.6 ± 0.0	2.8 ± 0.0	MS, RI
cis-α-Bisabolene	1545	0.1 ± 0.1	0.2 ± 0.1	0.4 ± 0.0	0.6 ± 0.0	MS, RI
(*E*)-Nerolidol	1568	0.4 ± 0.0	0.5 ± 0.3	0.4 ± 0.0	0.3 ± 0.0	MS, RI, std
Spathulenol	1579	1.3 ± 0.1	0.2 ± 0.1	0.7 ± 0.0	0.4 ± 0.0	MS, RI, std
Caryophyllene oxide	1583	–	–	0.7 ± 0.0	0.1 ± 0.0	
Humulene epoxide II	1601	0.4 ± 0.0	0.1 ± 0.1	0.4 ± 0.0	0.1 ± 0.0	MS, RI
β-Atlantol	1608	–	–	0.4 ± 0.0	0.1 ± 0.0	
α-Acorenol	1634	0.3 ± 0.2	0.2 ± 0.1	–	–	MS, RI
α-Cadinol	1656	0.1 ± 0.1	0.2 ± 0.1	–	–	MS, RI
β-Bisabolol	1674	0.1 ± 0.0	0.2 ± 0.1	–	–	MS, RI
α-bisabolol	1687	1.1 ± 0.5	0.4 ± 0.2	0.1 ± 0.0	0.1 ± 0.0	MS, RI, std
11-α*H*-Himachal-4-en-1-β-ol	1699	6.2 ± 0.3	10.1 ± 1.9	2.0 ± 0.1	1.7 ± 0.1	MS, RI
Mint sulfide	1740	t	t	–	–	MS, RI
Benzyl benzoate	1763	t	t	–	–	MS, RI
Tetradecanoic acid	1770	t	t	–	–	MS, RI
α-Bisabolol acetate	1795	–	–	0.1 ± 0.0	t	
Octadecane	1800	–	–	t	–	
Hexahydrofarnesyl acetone	1845	1.2 ± 0.0	0.2 ± 0.1	0.6 ± 0.0	0.1 ± 0.0	MS, RI, std
Benzyl salicylate	1862	t	–	–	–	MS, RI
Nonadecane	1900	–	–	0.1 ± 0.1	0.2 ± 0.0	
(5*E*,9*E*)-Farnesyl acetone	1916	0.1 ± 0.1	–	–	–	MS, RI
Farnesyl acetone	1927	–	–	0.1 ± 0.0	–	

(*continued overleaf*)

Table 2. (*Continued*)

Component[b]	RI[c]	HD[a] Dry	HD[a] Fresh	SPME[a] Dry	SPME[a] Fresh	Identification methods[d]
Hexadecanoic acid	1965	0.4 ± 0.1	t	–	–	MS, RI, std
Heneicosane	2100	0.6 ± 0.2	0.6 ± 0.4	–	–	MS, RI, std
Docosane	2200	t	t	–	–	MS, RI, std
Tricosane	2300	0.3 ± 0.0	0.2 ± 0.1	–	–	MS, RI, std
Tetracosane	2400	0.2 ± 0.0	0.1 ± 0.0	–	–	MS, RI, std
Pentacosane	2500	0.9 ± 0.2	0.6 ± 0.2	–	–	MS, RI, std
Hexacosane	2600	0.2 ± 0.0	0.1 ± 0.1	–	–	MS, RI, std
Heptacosane	2700	1.8 ± 0.6	1.0 ± 0.4	–	–	MS, RI, std
Octacosane	2800	0.2 ± 0.1	0.1 ± 0.0	–	–	MS, RI, std
Nonacosane	2900	1.0 ± 0.4	0.4 ± 0.2	–	–	MS, RI, std
Triacontane	3000	0.6 ± 0.3	0.2 ± 0.1	–	–	MS, RI, std
Grouped compounds (%)						
Terpenoids		83.3	82.7	90.7	93.7	
Monoterpenes hydrocarbons		14.2	11.5	1.8	1.3	
Oxygenated monoterpenes		8.9	9.4	6.7	5.7	
Sesquiterpenes hydrocarbons		48.5	49.4	76.4	83.4	
Oxygenated sesquiterpenes		11.1	12.2	5.4	3.0	
C_{13}-Norisoprenoids		0.6	0.2	0.5	0.2	
Aliphatics		14.4	16.5	4.7	3.8	
Alcohols		3.4	6.0	1.7	1.6	
Esters		3.5	3.4	2.5	1.4	
Aldehydes		1.2	2.6	–	–	
Acids		0.4	t	–	–	
Alkanes		5.9	3.5	0.1	0.2	
Aromatics		1.6	0.7	0.3	0.3	
Sulfur-containing compounds		t	t	–	–	
Total components		97	97	76	66	
Total identified (%)		99.3	99.0	95.8	97.8	

[a] Values, expressed in percentages, are means ± standard error of triplicate measurements. They were obtained at FID by peak area normalisation calculating the relative response factor (see Table 1).
[b] Compounds belonging to each class are listed in order of their elution from a HP-5 column.
[c] RI, retention indices as determined on HP-5 column using homologous series of C8-C30 alkanes.
[d] Identification methods: MS, by comparison of the mass spectrum with those of the computer mass libraries Wiley, NIST 05 and ADAMS; RI, by comparison of RI with those reported from literature;[16–17] std, by comparison of the retention time, mass spectrum and retention index of authentic standard.
t, traces (mean value below 0.1%); – , not detected.

that volatiles extracted by SPME were represented mainly by terpenoids (90.7–93.7%), with sesquiterpene hydrocarbons being the most abundant (76.4–83.4%). In fact, the major compound of this fraction, (*Z*,*E*)-α-farnesene, showed higher percentages (27.3–33.9%) with respect to those of essential oils (11.5–14.9%). Monoterpenes fraction resulted more abundant in the essential oils in comparison to headspace, with hydrocarbons more prevalent (11.5–14.2% vs. 1.3–1.8%, respectively) with respect to oxygenated ones (8.9–9.4% vs. 5.7–6.7%, respectively).

Finally, essential oils contained more aliphatics compounds with respect to headspace of plant material (14.4–16.5% vs. 3.8–4.7%, respectively). In particular, owing to the high temperatures and hydrolytic reactions occurring during hydrodistillation, essential oils contained higher amount of alkanes with respect to headspace of the plant material (3.5–5.9% vs. 0.1–0.2%).

The major volatile (*Z*,*E*)-α-farnesene is one of the two naturally occurring stereoisomers of α-farnesene, an acyclic sesquiterpene hydrocarbon that was found in the coating of apples,[19] and other pomoidea fruits. The two stereoisomers are responsible for the characteristic green apple odour. They have been detected in apple distillates where are responsible for an important 'apple-like quality' scent and, generally, for a 'fresh floral terpenyl topnote'.[20–22] They are used also for enhancing the aroma or taste of foodstuffs, chewing gums, medicinal products and toothpastes.[23] Being the major volatiles contributing to the scent of gardenia, they are used also in perfumery to enhance the aroma of perfume compositions, colognes and perfumed articles. Noteworthy is the occurrence in the oil of the sesquiterpene hydrocarbon zingiberene (2.1–2.6%), which is the major component of commercially available oil derived from rhizomes of the ginger plant *Zingiber officinale* Roscoe, widely used in cosmetics and fragrances. This compound was detected in many Lamiaceae of the Mediterranean area and contributes to the peculiar fragrance of the plants which are used as flavouring agent for white wines.[24] In addition, it is interesting to note the pres-

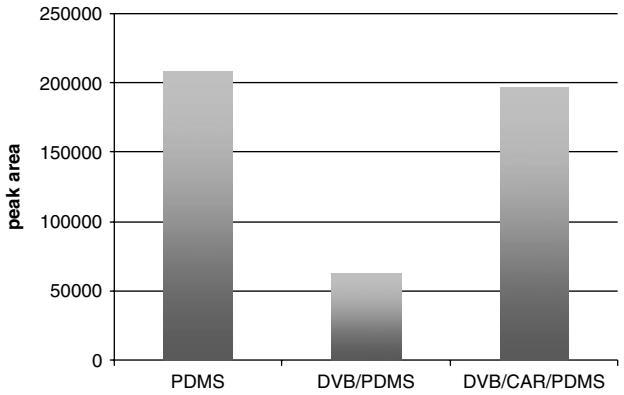

Figure 1. Uptake of volatiles, as sum of the peak areas, by three types of SPME fibre coating. Data obtained by GC/FID analysis.

ence in the oils of small amounts of some norisoprenoids, such as β-cyclocitral, dihydroedulan I, theaspirane A, theaspirane B, (*E*)-β-damascenone and (*E*)-β-ionone. These are C13 substances deriving from the degradation of carotenoid molecules,[25] and also from the hydrolysis of glucoside molecules.[26] Some norisoprenoids, such as (*E*)-β-damascenone and (*E*)-β-ionone, have been identified in wine.[27] The former is related to flowery, sweet and fruity notes, while the latter supplies an aroma of violets. The norisoprenoids have an important sensorial impact on wine aroma as they have very low olfactory perception thresholds. Therefore, they are useful as odour-modifying ingredients for manufacturing perfumes and perfumed products, and as flavour-modifying ingredients for the manufacture of artificial flavours for flavouring foodstuffs, animal feeds, beverages, pharmaceutical preparations and tobacco products. Another contribution to the characteristic fragrance of the plant came from 1-octen-3-ol (2.9–4.8%), mushroom aromatic compound, which is reported to be also an aroma component in several food products and beverages.[28] In addition, we also detected esters of salicylic acid, molecule involved in the plant defence system against pathogens.

In conclusion, the particular aromatic profile of the essential oils supports the use of *T. flavum* subsp. *flavum* in many manufactures as flavouring agent.

Solid-phase micro-extraction analysis

SPME is a valid alternative to HD; it has been used routinely in combination with GC and GC/MS, and successfully applied to a wide variety of compounds, especially for the extraction of volatile and semi-volatile organic compounds from environmental, biological and food samples.

In this study, three fibres, polydimethylsiloxane (PDMS, 100 µm), polydimethylsiloxane–divinylbenzene (PDMS/DVB, 65 µm) and

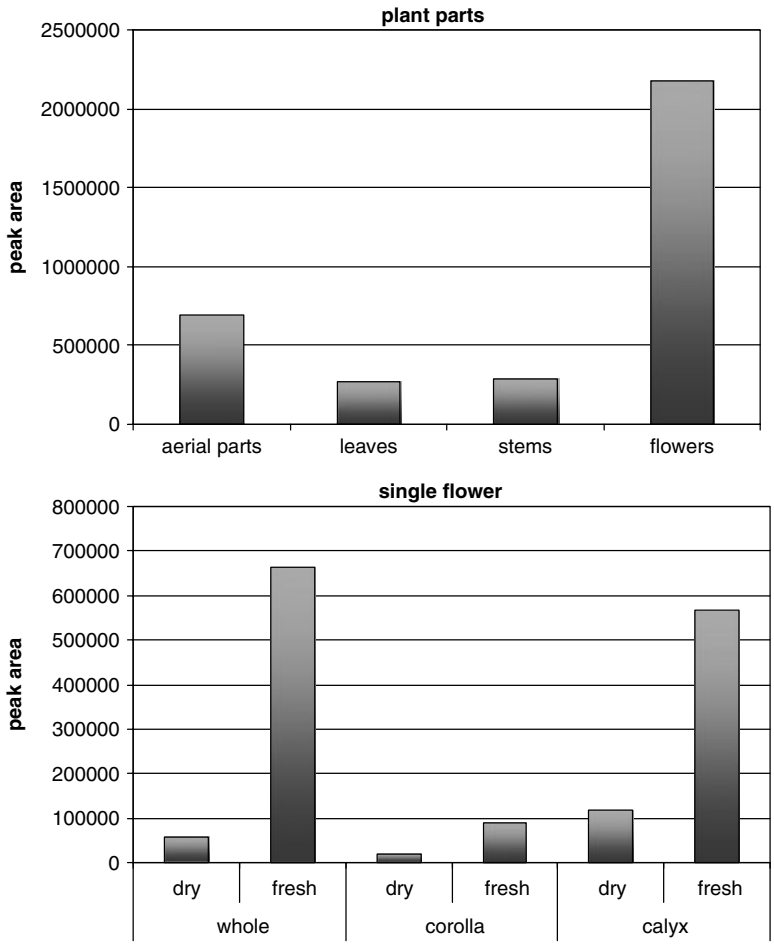

Figure 2. Emission of volatiles, on the basis of the sum of peak areas of SPME/GC/FID chromatograms, in different plant parts (dry) and within a single flower of *Teucrium flavum* susbsp. *flavum*. Values are average of three determinations.

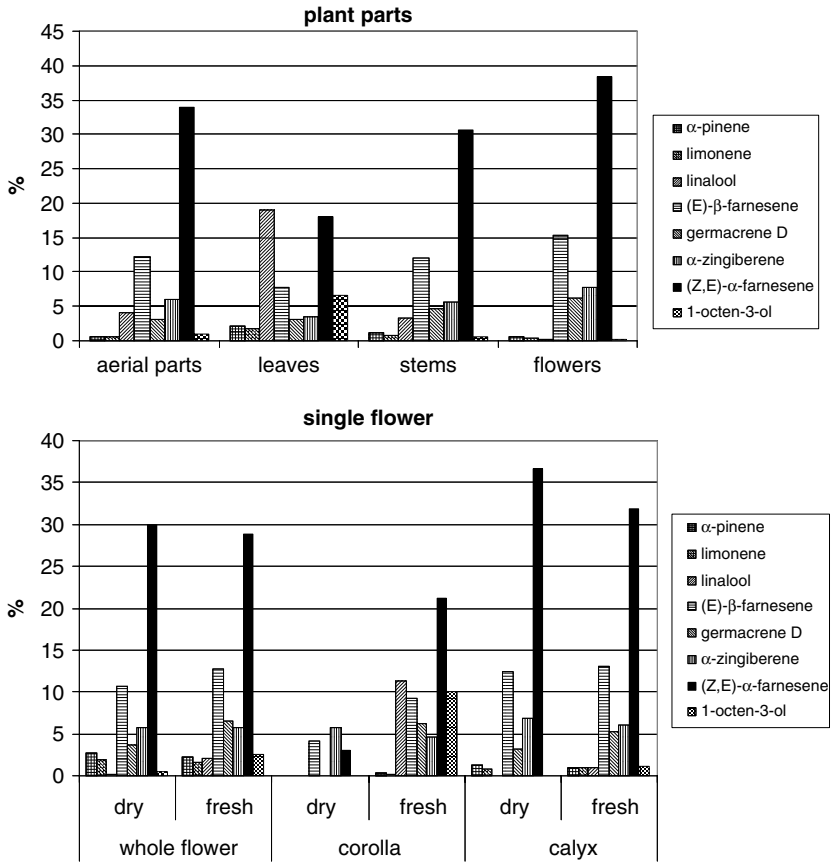

Figure 3. SPME/GC/FID analysis: percentages (mean values) of some aromatic compounds in different parts and within a single flower of *Teucrium flavum* subsp. *flavum*.

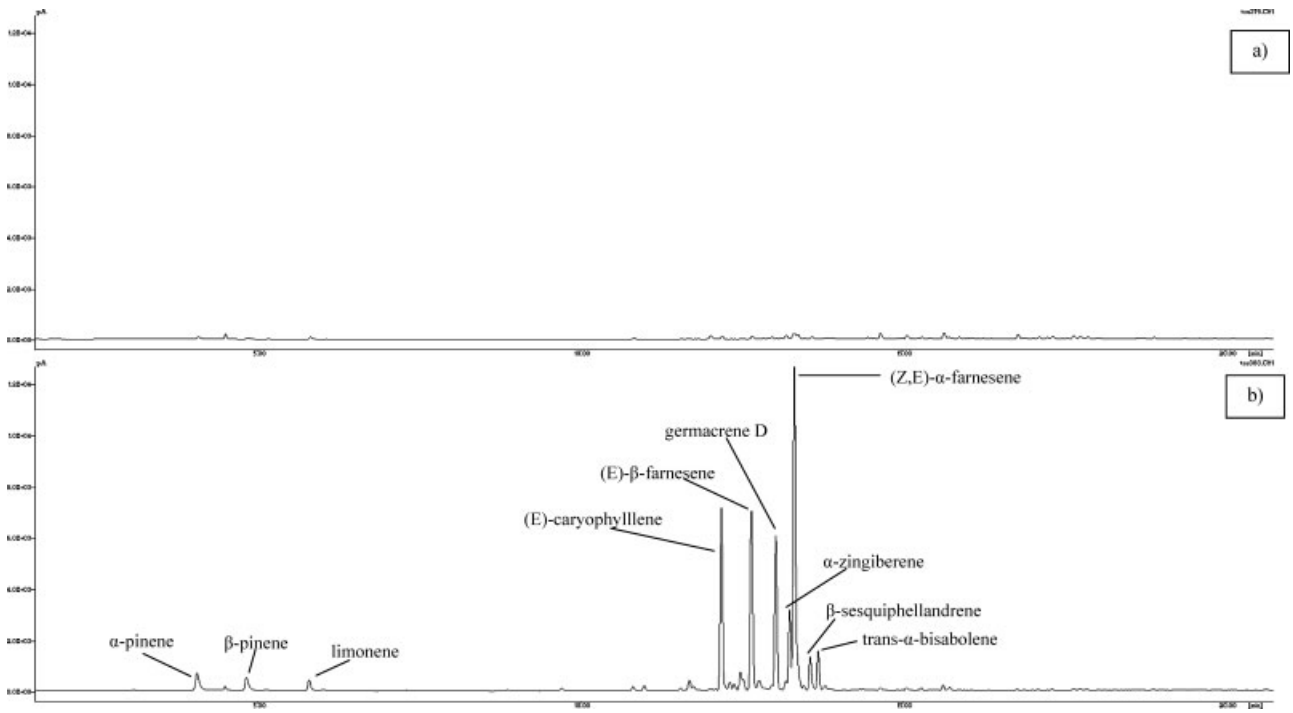

Figure 4. SPME/GC/FID chromatograms of a dry single whole flower of *Teucrium flavum* subsp. *flavum*. (a) Whole flower with joined calyx and corolla; (b) the same flower with the calyx manually separated from the corolla.

Table 3. SPME/GC/FID analysis: percentage composition[a] of volatiles in different plant parts and within a single flower of *Teucrium flavum* subsp. *flavum*

		Flowering aerial parts		Leaves	Stems	Flowers	Single flower						
							Whole		Corolla		Calyx		
Component[b]	RI[c]	Dry	Fresh	Dry	Dry	Dry	Dry (10.4 mg)	Fresh (37.1 mg)	Dry (4.0 mg)	Fresh (26.3 mg)	Dry (6.1 mg)	Fresh (12.4 mg)	Identification Methods[d]
(2*E*)-Hexenal	848	0.1 ± 0.0	0.3 ± 0.0	1.1 ± 0.0	–	t	–	0.4 ± 0.1	–	1.0 ± 0.5	–	0.4 ± 0.1	MS,RI
n-Hexanol	865	0.7 ± 0.1	0.4 ± 0.0	2.4 ± 0.1	0.7 ± 0.0	0.1 ± 0.0	–	0.6 ± 0.0	–	2.5 ± 0.5	–	0.5 ± 0.1	MS,RI
α-Pinene	935	0.6 ± 0.0	0.4 ± 0.1	2.1 ± 0.1	1.2 ± 0.1	0.5 ± 0.0	2.7 ± 0.8	2.3 ± 0.4	–	0.3 ± 0.0	1.2 ± 0.0	0.9 ± 0.1	MS,RI,std
Benzaldehyde	955	0.1 ± 0.0	t	–	0.1 ± 0.0	t	–	t	–	–	–	t	MS,RI,std
β-Pinene	977	0.5 ± 0.0	0.3 ± 0.1	1.6 ± 0.1	1.0 ± 0.0	0.3 ± 0.0	1.9 ± 0.5	1.6 ± 0.2	–	0.2 ± 0.0	0.8 ± 0.6	0.9 ± 0.0	MS,RI,std
1-Octen-3-ol	980	1.0 ± 0.0	1.2 ± 0.0	6.6 ± 0.0	0.6 ± 0.0	0.2 ± 0.0	0.4 ± 0.0	2.5 ± 0.6	–	10.1 ± 3.4	–	1.1 ± 0.2	MS,RI,std
Myrcene	988	0.1 ± 0.0	0.1 ± 0.0	0.6 ± 0.1	0.1 ± 0.0	t	0.2 ± 0.0	0.5 ± 0.1	–	–	–	0.2 ± 0.1	MS,RI,std
Limonene	1028	0.5 ± 0.0	0.3 ± 0.0	1.7 ± 0.0	0.7 ± 0.1	0.3 ± 0.0	1.4 ± 0.0	2.0 ± 0.5	–	0.2 ± 0.0	0.5 ± 0.3	0.6 ± 0.1	MS,RI,std
(*Z*)-β-Ocimene	1035	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	t	0.3 ± 0.0	0.9 ± 0.5	–	–	–	0.5 ± 0.1	MS,RI
(*E*)-β-Ocimene	1044	t	t	0.1 ± 0.0	–	t	–	0.4 ± 0.0	–	0.2 ± 0.0	–	0.1 ± 0.1	MS,RI
3,5-Octadien-2-one	1084	t	–	0.1 ± 0.0	–	t	–	t	–	0.2 ± 0.0	–	–	MS,RI
Linalool	1098	4.0 ± 0.2	3.4 ± 0.0	19.0 ± 0.4	3.3 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	2.1 ± 0.6	–	11.3 ± 3.3	–	0.9 ± 0.3	MS,RI,std
Nonanal	1100	t	0.2 ± 0.0	t	t	0.1 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	–	t	–	0.4 ± 0.0	MS,RI
n-Amyl isovalerate	1109	0.4 ± 0.0	t	1.7 ± 0.0	0.7 ± 0.0	t	0.6 ± 0.0	0.3 ± 0.0	–	t	–	0.2 ± 0.0	MS,RI
1-Octen-3-yl acetate	1112	0.2 ± 0.0	0.2 ± 0.0	1.2 ± 0.0	0.5 ± 0.0	0.2 ± 0.0	–	0.3 ± 0.1	–	0.4 ± 0.2	–	0.1 ± 0.0	MS,RI
3-Octanol, acetate	1120	1.0 ± 0.1	0.1 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.4 ± 0.0	0.1 ± 0.0	–	–	0.6 ± 0.0	0.1 ± 0.1	MS,RI
trans-Pinocarveol	1139	0.2 ± 0.0	–	0.3 ± 0.0	0.2 ± 0.0	t	0.4 ± 0.0	–	–	–	0.2 ± 0.0	–	MS,RI
Pinocarvone	1162	0.1 ± 0.0	–	0.3 ± 0.0	0.2 ± 0.0	t	0.3 ± 0.0	–	–	–	0.2 ± 0.0	–	MS,RI
Butanoic acid, hexyl ester	1190	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.7 ± 0.0	0.2 ± 0.0	–	–	0.2 ± 0.0	0.1 ± 0.0	MS,RI
Methyl salicylate	1194	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	0.5 ± 0.0	0.1 ± 0.0	–	0.4 ± 0.0	0.3 ± 0.0	0.1 ± 0.1	MS,RI
Myrtenol	1196	t	t	t	–	–	–	t	–	–	–	t	MS,RI,std
Decanal	1202	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	t	0.4 ± 0.0	t	–	0.5 ± 0.2	–	–	MS,RI
Verbenone	1205	0.1 ± 0.0	–	–	–	t	–	–	–	–	0.2 ± 0.0	–	MS,RI,std
β-Cyclocitral	1220	0.1 ± 0.0	–	0.3 ± 0.0	t	t	–	–	–	–	–	–	MS,RI
n-Hexyl 2-methyl butanoate	1233	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	–	–	0.1 ± 0.0	0.1 ± 0.0	MS,RI
cis-3-Hexenyl isovalerate	1239	t	0.1 ± 0.0	–	0.1 ± 0.0	t	–	0.1 ± 0.0	–	–	–	0.1 ± 0.0	MS,RI

(*continued overleaf*)

Table 3. (*Continued*)

Component[b]	RI[c]	Flowering aerial parts		Leaves	Stems	Flowers	Single flower						Identification Methods[d]
							Whole		Corolla		Calyx		
		Dry	Fresh	Dry	Dry	Dry	Dry (10.4 mg)	Fresh (37.1 mg)	Dry (4.0 mg)	Fresh (26.3 mg)	Dry (6.1 mg)	Fresh (12.4 mg)	
Hexyl isovalerate	1241	0.1 ± 0.0	t	0.1 ± 0.0	–	t	–	0.1 ± 0.0	–	–	0.2 ± 0.2	0.1 ± 0.0	MS,RI
Carvone	1244	t	t	–	–	–	–	t	–	–	–	–	MS,RI,std
Isoamyl hexanoate	1246	0.3 ± 0.0	0.4 ± 0.0	1.1 ± 0.0	1.5 ± 0.1	0.2 ± 0.0	2.5 ± 0.5	0.6 ± 0.2	–	–	–	0.5 ± 0.2	MS,RI
(2*E*)-Decenal	1260	t	–	–	–	–	–	–	–	–	–	–	MS,RI
trans-Linalool oxide acetate (pyranoid)	1289	1.8 ± 0.0	2.0 ± 0.1	1.3 ± 0.0	1.3 ± 0.0	1.1 ± 0.0	1.9 ± 0.6	1.1 ± 0.3	–	0.5 ± 0.0	0.8 ± 0.2	0.7 ± 0.2	MS,RI
Undecanal	1305	t	–	–	0.1 ± 0.0	t	–	–	–	–	–	–	MS,RI
Isoamyl heptanoate	1316	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	0.8 ± 0.0	0.1 ± 0.0	0.5 ± 0.0	0.1 ± 0.0	–	–	–	0.1 ± 0.0	MS,RI
Methyl geranate	1320	0.3 ± 0.0	0.3 ± 0.0	0.5 ± 0.1	0.7 ± 0.2	0.2 ± 0.0	2.4 ± 2.3	0.1 ± 0.1	23.6 ± 4.9	1.1 ± 0.3	1.2 ± 0.6	0.4 ± 0.1	MS,RI
Hexyl tiglate	1322	0.1 ± 0.0	0.1 ± 0.0	–	0.1 ± 0.0	0.1 ± 0.0	–	0.1 ± 0.0	–	–	–	0.1 ± 0.0	MS,RI
α-Longipinene	1350	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	–	–	–	0.1 ± 0.0	MS,RI,std
Eugenol	1356	t	t	–	–	t	–	–	–	–	–	–	MS,RI,std
α-Copaene	1374	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	–	–	0.1 ± 0.1	0.1 ± 0.0	MS,RI,std
Isoledene	1375	t	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	t	t	0.1 ± 0.0	–	–	0.7 ± 0.0	t	MS,RI
Geranyl acetate	1379	0.1 ± 0.0	t	0.1 ± 0.0	t	0.1 ± 0.0	–	0.1 ± 0.0	–	0.4 ± 0.0	–	0.1 ± 0.0	MS,RI
β-Bourbonene	1389	1.6 ± 0.3	0.9 ± 0.0	1.5 ± 0.0	2.0 ± 0.0	1.2 ± 0.0	0.7 ± 0.1	0.6 ± 0.1	–	0.4 ± 0.1	0.5 ± 0.3	0.4 ± 0.1	MS,RI
7-epi-Sesquithujene	1392	0.7 ± 0.0	0.6 ± 0.0	0.4 ± 0.0	0.7 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.5 ± 0.1	–	0.4 ± 0.0	0.4 ± 0.0	0.5 ± 0.2	MS,RI
Sesquithujene	1405	0.1 ± 0.0	0.2 ± 0.0	–	0.3 ± 0.0	0.1 ± 0.0	0.6 ± 0.2	0.1 ± 0.0	2.1 ± 0.4	–	–	0.1 ± 0.0	MS,RI
(*E*)-Caryophyllene	1420	5.1 ± 0.0	8.4 ± 0.0	2.2 ± 0.0	3.8 ± 0.0	4.0 ± 0.0	1.6 ± 0.9	7.8 ± 0.6	–	5.4 ± 0.4	3.1 ± 1.1	7.5 ± 0.6	MS,RI,std
β-Copaene	1430	0.6 ± 0.0	0.8 ± 0.0	0.4 ± 0.0	0.8 ± 0.1	0.5 ± 0.0	0.3 ± 0.0	0.5 ± 0.1	–	0.4 ± 0.0	0.3 ± 0.1	0.4 ± 0.2	MS,RI
trans-α-Bergamotene	1433	0.7 ± 0.0	0.5 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.7 ± 0.0	0.5 ± 0.1	0.5 ± 0.0	–	0.3 ± 0.0	0.5 ± 0.0	0.5 ± 0.1	MS,RI
(*Z*)-β-Farnesene	1442	2.2 ± 0.0	1.6 ± 0.0	1.2 ± 0.0	1.8 ± 0.0	2.4 ± 0.0	1.8 ± 0.2	1.6 ± 0.1	–	1.1 ± 0.1	1.8 ± 0.1	1.7 ± 0.2	MS,RI
Aromadendrene	1443	1.2 ± 0.0	0.9 ± 0.0	0.7 ± 0.0	1.2 ± 0.0	1.1 ± 0.0	1.0 ± 0.1	0.9 ± 0.1	–	0.6 ± 0.0	0.9 ± 0.2	0.8 ± 0.1	MS,RI,std
α-Humulene	1455	t	t	t	t	t	t	t	–	0.3 ± 0.0	t	t	MS,RI,std
(*E*)-β-Farnesene	1456	12.3 ± 0.1	13.3 ± 0.2	7.7 ± 0.1	12.1 ± 0.0	15.4 ± 0.0	10.6 ± 1.5	12.7 ± 1.1	4.1 ± 0.3	9.2 ± 1.0	12.5 ± 1.5	13.1 ± 4.4	MS,RI
allo-Aromadendrene	1459	1.0 ± 0.0	1.1 ± 0.1	0.6 ± 0.0	1.0 ± 0.0	0.6 ± 0.0	0.8 ± 0.1	0.7 ± 0.2	3.1 ± 0.2	0.9 ± 0.0	0.6 ± 0.2	0.6 ± 0.2	MS,RI,std

Table 3. (*Continued*)

Component[b]	RI[c]	Flowering aerial parts		Leaves	Stems	Flowers	Single flower						Identification Methods[d]
							Whole		Corolla		Calyx		
		Dry	Fresh	Dry	Dry	Dry	Dry (10.4 mg)	Fresh (37.1 mg)	Dry (4.0 mg)	Fresh (26.3 mg)	Dry (6.1 mg)	Fresh (12.4 mg)	
ar-Curcumene	1478	0.5 ± 0.0	0.2 ± 0.1	0.3 ± 0.0	0.5 ± 0.0	0.3 ± 0.1	0.5 ± 0.2	0.2 ± 0.0	2.7 ± 0.2	t	0.4 ± 0.1	0.2 ± 0.0	MS,RI
Amorpha-4,7(11)-diene	1479	0.3 ± 0.0	0.4 ± 0.1	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.5 ± 0.1	0.3 ± 0.0	–	0.3 ± 0.1	0.3 ± 0.0	0.4 ± 0.1	MS,RI
Germacrene D	1485	3.1 ± 0.0	13.2 ± 0.1	3.1 ± 0.0	4.7 ± 0.1	6.2 ± 0.1	3.7 ± 1.1	6.6 ± 3.3	–	6.2 ± 0.5	3.2 ± 0.6	5.2 ± 3.8	MS,RI,std
(*E*)-β-Ionone	1489	0.4 ± 0.0	0.2 ± 0.0	1.3 ± 0.0	0.6 ± 0.0	0.1 ± 0.0	0.4 ± 0.3	0.1 ± 0.0	2.0 ± 0.4	0.3 ± 0.0	–	0.1 ± 0.0	MS,RI,std
α-Zingiberene	1495	6.0 ± 0.0	6.5 ± 0.0	3.4 ± 0.0	5.7 ± 0.1	7.7 ± 0.2	5.8 ± 0.7	5.7 ± 0.4	5.7 ± 1.9	4.6 ± 1.0	6.9 ± 0.7	6.0 ± 2.5	MS,RI,std
(*Z*,*E*)-α-Farnesene	1498	33.9 ± 0.2	27.3 ± 0.4	18.1 ± 0.2	30.7 ± 0.3	38.5 ± 0.1	30.0 ± 1.4	28.9 ± 2.8	3.1 ± 0.5	21.2 ± 4.3	36.7 ± 4.4	31.9 ± 2.5	MS,RI
β-Bisabolene	1506	0.5 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	0.8 ± 0.1	0.1 ± 0.0	1.4 ± 0.8	0.5 ± 0.0	7.0 ± 3.2	0.9 ± 0.1	0.9 ± 0.2	0.7 ± 0.1	MS,RI
β-Curcumene	1515	t	t	t	t	t	3.0 ± 0.6	0.7 ± 0.1	t	0.9 ± 0.0	t	0.8 ± 0.1	MS,RI
δ-Cadinene	1523	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.1	–	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.1	MS,RI
β-Sesquiphellandrene	1524	2.8 ± 0.0	2.6 ± 0.0	1.7 ± 0.0	2.7 ± 0.0	3.4 ± 0.0	2.7 ± 0.3	2.6 ± 0.2	1.4 ± 0.0	2.2 ± 0.5	3.5 ± 0.4	3.1 ± 0.1	MS,RI
(*E*)-γ-Bisabolene	1530	t	t	t	t	0.3 ± 0.0	–	0.1 ± 0.0	–	t	–	0.1 ± 0.0	MS,RI
trans-α-Bisabolene	1535	2.6 ± 0.0	2.8 ± 0.0	1.4 ± 0.0	2.8 ± 0.1	3.9 ± 0.0	3.0 ± 0.4	3.2 ± 0.4	1.0 ± 0.3	2.6 ± 0.7	4.4 ± 0.8	3.8 ± 0.3	MS,RI
cis-α-Bisabolene	1542	0.4 ± 0.0	0.6 ± 0.0	0.3 ± 0.1	0.5 ± 0.1	0.1 ± 0.0	0.4 ± 0.1	0.5 ± 0.0	1.8 ± 0.2	0.3 ± 0.1	0.6 ± 0.1	0.6 ± 0.0	MS,RI
(*E*)-Nerolidol	1561	0.4 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.5 ± 0.0	0.1 ± 0.1	0.4 ± 0.1	0.3 ± 0.0	–	0.3 ± 0.1	0.5 ± 0.1	0.6 ± 0.0	MS,RI,std
Spathulenol	1577	0.7 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	0.6 ± 0.0	0.1 ± 0.1	0.5 ± 0.1	0.2 ± 0.0	–	0.4 ± 0.0	0.5 ± 0.2	0.4 ± 0.1	MS,RI,std
Caryophyllene oxide	1583	0.7 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.5 ± 0.0	0.4 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	–	0.1 ± 0.0	0.4 ± 0.2	0.1 ± 0.0	MS,RI,std
β-Atlantol	1608	0.4 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.4 ± 0.0	0.1 ± 0.0	0.7 ± 0.3	0.1 ± 0.0	2.2 ± 0.6	0.3 ± 0.1	0.5 ± 0.1	0.1 ± 0.0	MS,RI
Humulene epoxide II	1610	0.4 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.4 ± 0.0	0.1 ± 0.0	–	0.1 ± 0.0	–	0.2 ± 0.0	0.2 ± 0.2	0.1 ± 0.0	MS,RI
α-Bisabolol	1688	0.1 ± 0.0	0.1 ± 0.0	–	0.1 ± 0.0	0.1 ± 0.0	–	0.1 ± 0.0	–	0.2 ± 0.0	0.7 ± 0.2	0.2 ± 0.0	MS,RI,std
11-α-*H*-Himachal-4-en-1-β-ol	1699	2.0 ± 0.1	1.7 ± 0.1	1.3 ± 0.1	2.2 ± 0.2	2.7 ± 0.1	2.6 ± 1.3	2.2 ± 0.1	7.2 ± 1.5	2.8 ± 0.4	3.0 ± 1.1	5.4 ± 0.3	MS,RI
α-Bisabolol acetate	1795	0.1 ± 0.0	t	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	–	t	–	–	0.2 ± 0.0	0.1 ± 0.0	MS,RI
Octadecane	1800	t	–	–	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.1	t	0.9 ± 0.0	–	0.1 ± 0.0	t	MS,RI,std
Hexahydrofarnesyl acetone	1845	0.6 ± 0.0	0.1 ± 0.0	1.6 ± 0.1	1.1 ± 0.1	t	1.9 ± 1.4	0.1 ± 0.0	16.0 ± 6.4	0.9 ± 0.3	0.3 ± 0.0	0.1 ± 0.0	MS,RI,std

(*continued overleaf*)

Table 3. (*Continued*)

Component[b]	RI[c]	Flowering aerial parts		Leaves	Stems	Flowers	Single flower						Identification Methods[d]
							Whole		Corolla		Calyx		
		Dry	Fresh	Dry	Dry	Dry	Dry (10.4 mg)	Fresh (37.1 mg)	Dry (4.0 mg)	Fresh (26.3 mg)	Dry (6.1 mg)	Fresh (12.4 mg)	
Nonadecane	1900	0.1 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.8 ± 0.0	0.3 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	MS,RI,std
Farnesyl acetone	1927	0.1 ± 0.0	–	0.2 ± 0.0	0.1 ± 0.0	–	–	–	–	–	–	–	MS,RI
Grouped compounds (%)													
Terpenoids		90.7	93.7	79.1	89.2	94.6	88.9	90.0	83.0	78.2	90.2	91.6	
Monoterpenes hydrocarbons		1.8	1.3	6.2	3.1	1.2	6.6	7.6	–	1.0	2.5	3.3	
Oxygenated monoterpenes		6.7	5.7	21.5	5.7	1.8	5.1	3.4	23.6	13.3	2.6	2.0	
Sesquiterpenes hydrocarbons		76.4	83.4	45.7	73.7	87.9	70.4	75.6	31.9	58.4	78.7	79.0	
Oxygenated sesquiterpenes		5.4	3.0	4.1	6.0	3.6	6.4	3.3	25.5	5.2	6.4	7.2	
C_{13}-Norisoprenoids		0.5	0.2	1.5	0.7	0.1	0.4	0.1	2.0	0.3	–	0.1	
Aliphatics		4.7	3.8	15.4	5.8	1.8	6.9	6.2	1.7	15.1	1.4	2.5	
Alcohols		1.7	1.6	9.0	1.3	0.3	0.4	3.2	–	12.6	–	1.6	
Esters		2.5	1.4	4.9	4.1	1.0	5.0	2.0	–	0.4	1.2	1.4	
Aldehydes and chetons		0.3	0.6	1.4	0.3	0.1	1.0	1.0	–	1.8	–	0.8	
Alkanes		0.1	0.2	0.1	0.1	0.3	0.5	0.1	1.7	0.3	0.2	0.1	
Aromatics		0.3	0.3	0.4	0.4	0.1	0.5	0.1	–	0.4	0.3	0.2	
Total components		76	66	66	67	72	54	68	18	49	47	65	
Total identified (%)		95.8	97.8	94.9	95.4	96.5	96.4	96.3	84.6	93.8	91.9	95.6	

[a] Values, expressed in percentages, are means ± standard error of triplicate measurements.
[b] Compounds belonging to each class are listed in order of their elution from a HP-5 column.
[c] RI, retention indices as determined on HP-5 column using homologous series of C8–C30 alkanes.
[d] Identification methods: MS, by comparison of the mass spectrum with those of the computer mass libraries Wiley, NIST 05 and ADAMS; RI, by comparison of RI with those reported from literature;[16,17] std, by comparison of the retention time, mass spectrum and retention index of authentic standard.
t, traces (mean value below 0.1%); – , not detected.

Stableflex divinylbenzene–carboxen–polydimethylsiloxane (DVB/CAR/PDMS, 50/30 μm) were evaluated for the analysis of the aroma components of *T. flavum* subsp. *flavum*. The fibre screening (Fig. 1) was based on the sum of the peak areas of all the volatiles detected in the matrix. The results confirmed that the PDMS fibre produced the best results for the investigated compounds. In particular, it has already been shown that PDMS fibre is the most appropriate for extraction of α-farnesene, which is the major volatile in the plant under study.[29]

SPME allowed the sampling of volatiles emitted by small amounts (30 mg) of different parts of the plant in a fast and easy manner. Moreover, the good concentration capability of this technique permitted the identifications of many compounds (76 different volatiles) (Table 3). Sesquiterpene hydrocarbons were the main class of volatiles in all investigated parts, showing a percentage ranging from 45.7% in leaves to 87.9% in flowers, with (*Z*,*E*)-α-farnesene (18.1–38.5%), (*E*)-β-farnesene (7.7–15.4%) and germacrene D (3.1–13.2%) the major components. As reported in Fig. 2, the highest contribute of volatiles, in terms of the sum of SPME/GC/FID peak areas, was given by flowers, that resulted the richest in sesquiterpene hydrocarbons (87.9%), such as (*Z*,*E*)-α-farnesene (38.5%), (*E*)-β-farnesene (15.4%), and α-zingiberene (7.7%); these components, as reported above, are volatiles contributing to the particular fragrance of the plant.

Among all plant parts investigated, leaves were the richest in monoterpenes (27.7%), with linalool (19.0%) as the most abundant, whilst they contained a lower amount of sesquiterpenes (49.8%). In addition, they revealed the highest amount of the mushroom-like flavour component 1-octen-3-ol (6.6%) (Fig. 3).

As resulted from SPME sampling (Table 3, Fig. 2), within a single flower, calyx afforded the major contribute in terms of volatiles in comparison to corolla. This could be due to the higher amount of secretory trichomes occurring on the sepals with respect to petals.[15] In particular, calyx contained a higher percentage of sesquiterpenes (85.1% and 86.2% in dry and fresh samples, respectively), with respect to corolla (57.4% and 63.6% in dry and fresh samples, respectively), which instead contained a higher amount of monoterpenes (14.3–23.6%). In all cases, volatiles decreased in number and quantitatively with the drying process, in particular in the corolla, where drying caused a loss of many components with respect to fresh material (18 vs. 49). Because of this, dry corolla resulted rich in the monoterpene ester methyl geranate (23.6%). Interestingly, fresh corolla was found to be the major source of linalool (11.3%) and of the aromatic C8 compound 1-octen-3-ol (10.1%) (Fig. 3). It is interesting to note that in a single dry whole flower, the emission of volatiles increased with the hand-separation of calyx from corolla. This is observable in Fig. 4, where the SPME/GC/FID chromatograms of a single flower are reported, in which calyx and corolla are naturally joined (Fig. 4a) or manually separated (Fig. 4b). This confirms the differences noticed in Fig. 2, where the volatile emission in a dry whole flower was lower than that in the dry calyx, and could be explained by the fact that the secretory trichomes responsible for volatile emissions are concentrated in the area of the flower where corolla is merged to calyx and that they released volatiles when they are separated.

CONCLUSIONS

In conclusion, HD permitted to obtain a higher number of volatiles (106 vs. 76) with respect to SPME, and showed a higher content in monoterpenes (20.9–23.1%) and alkanes (3.5–5.9%). On the other hand, with SPME extraction more sesquiterpenes (81.8–86.4% vs. 59.5–61.6%, respectively) were obtained, in particular the fruit-like component (*Z*,*E*)-α-farnesene (27.3–33.9%). SPME analysis showed different qualitative and quantitative emissions of volatiles between different plant parts and within a single flower. The major contribution to the aroma of the plant was given by flowers, while their fragrance seemed to depend mainly on the volatiles emitted from sepals of calyx, which included the majority of compounds detected in the whole flower (47 out of 54, and 65 out of 68 in dry and fresh samples, respectively). However, the corolla was the major source of some volatile compounds, such as linalool and 1-octen-3-ol.

The particular fruit-like aroma of the plant confirms the usefulness of the species as natural flavouring agent in wines, beers, bitters and liqueurs, and suggests also other applications, mainly as aroma and taste enhancer in food processing. SPME findings show that collection of this plant as a flavouring ingredient may be conducted also after the end of flowering, when the corollas fall and only the sepals, which are responsible for the emission of the apple-like aroma, remain on the twigs. Therefore, SPME proved to be a very useful technique, which permits a choice in the part of the plant which is the best source of a specific fragrance, and therefore the best way of sampling in industrial applications of aromatic plants can be established.

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