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Received June 8, 2010
Revised October 28, 2010
Accepted November 25, 2010

Research Article

A study on the essential oil of *Ferulago campestris*: How much does extraction method influence the oil composition?

The essential oil of different parts of *Ferulago campestris* (Bess.) collected in Sicily has been extracted by microwave-assisted hydrodistillation (MAHD) and by classic hydrodistillation (HD). A comparative qualitative–quantitative study on the composition of the oils was carried out. A total of 100 compounds were identified in the oils obtained by MAHD, whereas 88 compounds characterized the HD oils. The most prominent components were, in all different parts of *F. campestris* and in both extraction methods, 2,4,5-trimethylbenzaldehyde and 2,4,6-trimethylbenzaldehyde isomers; the latter was not previously found. The attempt to evaluate where the oil components are located in all parts of the plant was carried out by means of a kinetic study. Then, electron microscopy observation on the different parts before and after MAHD and HD was performed.

Keywords: Apiaceae / Essential oils / *Ferulago campestris* / Kinetic of microwave extraction / Microwaves hydrodistillation
DOI 10.1002/jssc.201000411

1 Introduction

Microwave technology applied to organic chemistry as non-classical energy source has come to play a role for the extraction of secondary metabolites from natural products [1]. Hence, since the first studies by Ganzler et al. [2], in the last decade, essential oil extraction by microwaves has become an alternative, valid extraction method, in terms of reproducibility, time, yield, purity and costs.

In this subject, the interest of scientific community is shown on a great number of articles published in which are reported techniques such as microwave-assisted solvent extraction (MASE) [3, 4], microwave-assisted hydrodistillation (MAHD) [4–6], solvent-free microwave extraction (SFME) [7–10], vacuum microwave (VM) [11, 12], compressed air microwave distillation (CAMD) [13], microwave-accelerated steam distillation (MASD) [14], microwave hydro-diffusion and gravity (MHG) [15, 16] and finally microwave steam distillation (MSD) [17]. However, the composition and consequently the properties of the essential oils extracted through these or conventional methods have been found to vary depending on the method used [9, 10, 16, 18], as well as the aspect linked to the territory [19, 20].

Ferulago campestris (Besser) Grec., (*F. galbanifera* (Mill) Kock. = *Ferula ferulago* L.), commonly known as ''*Finocchiazzo*'', is an annual or perennial herb genus of the Apiaceae family with small flowers in simple or compound umbels. The genus comprises about 40 species widely distributed in the temperate zone of both hemispheres, especially in Central Asia and Mediterranean area. Some *Ferulago* species are used since ancient times in folk medicine for their sedative, tonic, digestive, aphrodisiac properties and have been used in the treatment of intestinal worms and hemorrhoids. Moreover, they are used against ulcers, snake bite, as well as headache and diseases of the spleen [21]. Previous phytochemical studies on *F. campestris* revealed the presence of monoterpene coumarins [22]; moreover, the composition of the essential oil from a plant growing wild in Turkey was investigated [20]. Recently, Maggi et al. [19] reported the chemical composition of essential oils obtained by classic hydrodistillation (HD) methods from flowers and leaves of *F. campestris* collected in two sites in central Italy. The results showed relevant differences compared with the chemical composition reported previously [20]. In particular, 2,4,5-trimethyl benzaldehyde, the most abundant component from Turkish oils, did not occur in the essential oils from central Italy. On the contrary, 2,3,4-trimethyl benzaldehyde (0.1–0.5%) and 2,3,6-trimethyl benzaldehyde (0.4–4.3%) were detected.

Therefore, in continuation to our interest in both extracting [9, 23] and studying properties of Madonie's

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Abbreviations: BA, benzyl aldehyde; **HD**, hydrodistillation; **MAHD**, microwave assisted hydrodistillation method; **MH**, monoterpene hydrocarbons; **OM**, oxygenated monoterpenes; **OS**, oxygenated sesquiterpenes; **SH**, sesquiterpene hydrocarbons

endemic plants [24], where a rich biodiversity is present, the aim of this study was to investigate the potential of MAHD for the extraction of essential oil from different parts (steams and leaves, flowers, roots and seeds) of *F. campestris* collected in Sicily. A comparison in terms of extraction time, yield, qualitative composition of extracts and morphological change with conventional HD method has also been made.

2 Materials and methods

2.1 Plant material

Fresh *F. campestris* was collected in Alimena in Madonie National Park (Sicily), s.l. 910 m, in May 2009. It was authenticated by the Botanic Department of University of Palermo with specimen number *Raimondo, Schimmenti & Scafidi*, PAL 07-621.

2.2 Microwave apparatus and procedure

The microwave apparatus was a SAIREM downstream microwave source working at 2.45 GHz [23, 25, 26]. The generator is connected with an insulator which avoids damage to the generator, a sample holder constituted by a rectangular waveguide connected to two cylindrical waveguides, and a water load to absorb transmitted power. The main characteristics of this exposure setup are the homogeneous field distribution inside the sample holder, the ability to measure the microwave power absorbed by the sample and the simplicity in use. A schematic representation of the experimental setup is shown in Fig. 1.

In a typical MAHD procedure, performed at atmospheric pressure, the dried and triturated aerial parts constituted by steams and leaves (20 g), or flowers (20 g), or roots (20 g) or seeds (20 g) of *F. campestris* with 50 mL of water were placed in the glass cylinder, inserted in the waveguide and irradiated using a fix incident power of 200 W for 30 min. The non-perturbative optical fibre thermometer (Nortech Reflex-TP21M02) is introduced inside the glass cylinder to monitor the temperature during the treatment.

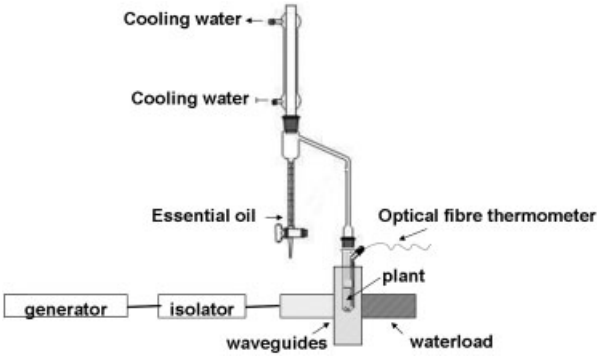

Figure 1. Schematic representation of the microwave apparatus used for MAHD.

A Clevenger refrigerator provided with a glass stopcock and a circulating water condenser is applied on the sample holder to collect the extracted essential oil. The energy is absorbed by the water causing a temperature increase distributed to all. After 2 min, the temperature system achieves 100°C and the water starts evaporating. Following condensation, the mixture essential oil–water is extracted with *n*-pentane. The organic phase is evaporated at 35°C under argon at atmospheric pressure and dried over anhydrous sodium sulphate. The essential oils are stored at 4°C under argon until used.

2.3 HD apparatus and procedure

The dried and triturated aerial parts constituted by steams and leaves (20 g), or flowers (20 g), or roots (20 g) or seeds (20 g) of *F. campestris* were submitted to HD for 3 h with Clevenger-type apparatus according to the standard procedure described in the *European Pharmacopoeia* [27], using *n*-pentane as solvent. The organic phase was evaporated at 35°C under argon at atmospheric pressure and dried over anhydrous sodium sulphate. The essential oils were stored at 4°C under argon until used.

2.4 GC

Analytical GC was carried out on a Perkin-Elmer Sigma 115 gas chromatograph fitted with an HP-5 MS capillary column (30 m × 0.25 mm id, 0.25 μm film thickness). Helium was the carrier gas (1 mL/min). Column temperature was initially kept at 40°C for 5 min, then gradually increased to 250°C at 2°C/min rate, held for 15 min and finally raised to 270°C at 10°C/min. Diluted samples (1:100 v/v, in *n*-hexane) of 1 μL were injected at 250°C, manually and in the splitless mode. Flame ionization detection (FID) was performed at 280°C. Analysis was also run by using a fused silica HP Innowax polyethylenglycol capillary column (50 m × 0.20 mm id, 0.20 μm film thickness).

2.5 GC-MS identification

GC-MS analysis was performed on an Agilent 6850 Ser. II apparatus, fitted with a fused silica HP-1 capillary column (30 m × 0.25 mm id, 0.33 μm film thickness), coupled to an Agilent Mass Selective Detector MSD 5973; ionization voltage 70 eV; electron multiplier energy 2000 V. Range of the mass charge ratio m/z was 29–450 and scan time was 1 s. GC conditions were as reported above; transfer line temperature, 295°C.

Table 1. Relative amount (%) and identification of the main components in the essential oil of *F. campestris* extracted by HD and MAHD method[a)]

Entry	Compound	GC Peak area (%)[b)]								RI[c)]	RI[d)]	Identification[e)]
		A_{HD}	A_{MAHD}	F_{HD}	F_{MAHD}	R_{HD}	R_{MAHD}	S_{HD}	S_{MAHD}			
1	α-Pinene	2.5	0.1	2.0	0.3	4.3	0.9	3.6	1.5	936	1075	Ri, MS, Co-GC
2	1,3,5-Trimethylbenzene								0.2	965	1230	Ri, MS, Co-GC
3	Sabinene	0.3		0.3		0.6				973	1132	Ri, MS, Co-GC
4	Pseudocumene	0.2		0.1	0.1	1.3	0.2	4.0	7.4	985	1249	Ri, MS
5	Myrcene	3.3	0.2	4.2	2.4					993	1174	Ri, MS, Co-GC
6	δ-3-Carene	0.5		1.8	0.4		*t*	0.6	*t*	1012	1157	Ri, MS
7	*p*-Cymene	12.0	5.3	14.8	12.0	0.2		6.3	4.7	1024	1278	Ri, MS, Co-GC
8	Limonene			0.6		0.2		0.5		1030	1203	Ri, MS, Co-GC
9	(*Z*)-β-ocimene	0.8		0.9	0.4					1038	1245	Ri, MS, Co-GC
10	(*E*)-β-ocimene	0.2		0.7	0.4					1049	1265	Ri, MS, Co-GC
11	γ-Terpinene	6.7	3.9	9.3	5.2	0.1		4.5		1057	1256	Ri, MS, Co-GC
12	Acetophenone			0.1						1065	1625	Ri, MS, Co-GC
13	Terpinolene	1.2	0.2	1.6	1.0				2.1	1086	1265	Ri, MS, Co-GC
14	*p*-Cymenene		1.7					1.3		1091	1429	Ri, MS
15	6-Camphenone	0.2		0.2	0.1			0.5		1093		Ri, MS
16	Linalool	0.1								1098	1553	Ri, MS, Co-GC
17	*trans*-*p*-Mentha-2,8-dien-1-ol	0.1					0.1			1119	1672	Ri, MS
18	α-Campholenal					0.3	0.1	0.7		1128	1487	Ri, MS
19	Chrysanthenone[f)]					0.2				1129	1524	Ri, MS
20	*allo*-Ocimene *cis*	0.6		0.8	0.3					1132	1382	Ri, MS
21	*trans*-Pinocarveol						*t*			1138	1665	Ri, MS
22	*cis*-Verbenol					0.1	0.2		0.1	1144	1663	Ri, MS
23	Eucarvone		1.2	0.4	0.4					1145	1465	Ri, MS
24	*trans*-Verbenol					0.1				1152	1683	Ri, MS
25	(*E*)-Chrysanthenol			0.3		0.4		1.8		1164	1684	Ri, MS
26	4-Ethyl benzaldehyde	0.2	0.2		0.1	0.1	0.6		0.4	1167	1734	Ri, MS
27	*p*-Cymen-8-ol	1.4		1.3		1.4	1.0	0.9	1.8	1185	1856	Ri, MS
28	Estragole						0.1		0.2	1195	1656	Ri, MS, Co-GC
29	Myrtenol		0.2							1196	1804	Ri, MS
30	Safranal	0.8	0.1	0.2	0.7	0.3	0.3			1201	1618	Ri, MS
31	*cis*-Verbenone	0.1				0.3				1208	1723	Ri, MS
32	*trans*-Carveol					0.1	0.2	0.2	0.1	1217	1845	Ri, MS
33	*trans*-Verbenone	0.2		0.1			0.2		0.1	1217	1725	Ri, MS
34	Nerol	0.3		0.6		0.2		1.4	0.1	1227	1798	Ri, MS, Co-GC
35	*p*-Anisaldehyde						0.1			1232	2015	Ri, MS
36	Cumin aldehyde		0.1		0.1				0.1	1232	1804	Ri, MS
37	Neral		0.3							1240	1692	Ri, MS
38	Piperitone		0.1							1252	1732	Ri, MS
39	Carvenone	0.5		0.6	0.2					1254	1682	Ri, MS
40	*cis*-Chrysanthenyl acetate		*t*	0.4	2.1	3.9	3.3	4.4	3.7	1257	1582	Ri, MS
41	*cis*-Verbenyl acetate[f)]	2.9		2.1						1276		Ri, MS
42	Bornyl acetate	0.1	0.1	0.1	0.1	0.1	0.1			1290	1597	Ri, MS
43	(*E,Z*)-2,4-Decadienal					0.1	0.1			1291	1779	Ri, MS
44	Thymol			0.4	0.7	0.2	*t*		*t*	1294	2198	Ri, MS, Co-GC

Table 1. Continued

Entry	Compound	GC			Peak area	(%)[b]				RI[c]	RI[d]	Identification[e]
		A_{HD}	A_{MAHD}	F_{HD}	F_{MAHD}	R_{HD}	R_{MAHD}	S_{HD}	S_{MAHD}			
45	*p*-Methoxy acetophenone	1.0			0.9	1.2	1.7	0.2	1.1	1302	1797	Ri, MS, Co-GC
46	2,4,5-Trimethylbenzaldehyde	38.6	2.3	2.6	3.9	6.0	5.7	29.1	5.2	1308	1895	Ri, MS, Co-GC
47	(*E,E*)-2,4-Decadienal					0.1				1315	1827	Ri, MS
48	Cuminyl acetate							0.4		1328		Ri, MS
49	2,4,6-Trimethylbenzaldehyde		33.2	29.6	43.2	49.2	22.4		51.2	1336	1928	Ri, MS
50	α-Cubebene			1.1	0.2					1352	1466	Ri, MS
51	Eugenol			0.1						1353	2186	Ri, MS, Co-GC
52	α-Longipinene						0.2			1355	1848	Ri, MS
53	α-Copaene		0.1							1377	1497	Ri, MS
54	β-Cubebene		0.2	0.2						1382	1547	Ri, MS
55	β-Elemene	0.5	3.3	1.4	1.6	0.3				1387	1600	Ri, MS
56	Eugenol methyl ether				0.1					1399	1958	Ri, MS, Co-GC
57	Cyperene					0.1				1401	1540	Ri, MS
58	β-Caryophyllene	9.4	5.0	1.6	1.7	3.6	4.3	2.2	0.1	1414	1612	
59	Widdrene			0.1	0.1					1433	1621	Ri, MS
60	α-Guaiene		0.1		0.1					1434	1527	Ri, MS
61	γ-Elemene		0.2	0.1	0.2	0.1				1435	1652	Ri, MS
62	(*E*)-β-Farnesene			0.1						1452	1673	Ri, MS
63	Geranyl acetone		0.5							1453	1867	Ri, MS
64	α-Humulene	0.1	0.7	0.3	0.4	0.2	0.1			1455	1689	Ri, MS
65	α-Muurolene		0.2							1455	1689	Ri, MS
66	γ-Selinene	0.1		0.1	*t*					1456	1687	Ri, MS
67	*allo*-Aromadendrene				*t*					1463	1661	Ri, MS
68	γ-Gurjunene			0.1						1472	1676	Ri, MS
69	β-Selinene	1.2	4.9	2.4	1.8	0.7	0.7	1.9	0.7	1475	1715	Ri, MS
70	Germacrene D	0.1	3.1	0.2	0.8		0.2			1477	1726	Ri, MS
71	γ-Muurolene	0.1		0.1	0.1					1478	1704	Ri, MS
72	*epi*-Bicyclosesquiphellandrene		0.5		0.1					1489	1734	Ri, MS
73	β-Guaiene		0.1							1490	1612	Ri, MS
74	α-Selinene	0.7	2.5	1.4	1.0	0.6	0.7	1.6	1.1	1498	1744	Ri, MS
75	γ-Cadinene	0.1	0.3							1515	1776	Ri, MS
76	δ-Cadinene	1.2	4.3	1.5	1.3	1.4	1.0	3.5	1.2	1526	1773	Ri, MS
77	α-Calacorene		1.1	0.3	0.4	0.2	0.2	2.9	0.9	1541	1918	Ri, MS
78	β-Calacorene		0.8	0.1	0.2		0.2	0.5	0.2	1550	1942	Ri, MS
79	*cis*-α-Copaen-8-ol	0.2	1.4		0.5		0.5		1.9	1553	2076	Ri, MS
80	(*E*)-Nerolidol	0.6	0.8	0.5	0.8	1.8	2.6	0.9	0.5	1560	2050	Ri, MS
81	Ledol								0.3	1565	2051	Ri, MS
82	Caryophyllene oxide	1.5	1.8	2.6	2.5	2.5	4.7	6.3	3.6	1579	2008	Ri, MS, Co-GC
83	Spathulenol	0.3	1.6					4.5	2.2	1580	2150	Ri, MS, Co-GC
84	Globulol								0.1	1587	2098	Ri, MS
85	Humulene epoxide II	0.3		0.4		0.3	0.6			1605	2071	Ri, MS
86	β-Oplopenone								0.1	1608	2098	Ri, MS
87	Isospathulenol	0.1		0.1		0.2	0.2	0.8	0.2	1635	2221	Ri, MS
88	T-Cadinol	0.2		0.1						1640	2185	Ri, MS
89	T-Muurolol		0.1			0.1				1641	2209	Ri, MS

Table 1. Continued

Entry	Compound	Peak area (%)[b]								RI[c]	RI[d]	Identification[e]
		A_{HD}	A_{MAHD}	GC F_{HD}	F_{MAHD}	R_{HD}	R_{MAHD}	S_{HD}	S_{MAHD}			
90	a $C_{15}H_{22}O$		0.4							1644		Ri, MS
91	α-Cadinol	0.9	0.3	0.3						1652	2255	Ri, MS
92	*cis*-α-Valerenol	0.5		0.5	0.9	0.1				1653	1872	Ri, MS
93	a $C_{15}H_{24}O$								0.2	1656		Ri, MS
94	Aristolenol		0.3		0.8		0.3			1658		Ri, MS
95	Cadalene	0.7	1.0	0.4				0.8	0.3	1677	2256	Ri, MS
96	(*E,E*)-Farnesol								0.1	1722	2369	Ri, MS
97	Benzyl benzoate	*t*		0.1						1762	2655	Ri, MS, Co-GC
98	1-Octadecene		0.2							1792		Ri, MS
99	Octadecane		*t*							1800	1800	Ri, MS, Co-GC
100	Neophytadiene		0.1							1809		Ri, MS
101	Hexahydrofarnesylacetone	0.1	1.1							1845	2131	Ri, MS
102	(*E,E*)-Farnesyl acetone		0.5							1918	2389	Ri, MS
103	Hexadecanoic acid methyl ester			0.1						1929	2208	Ri, MS, Co-GC
104	(*Z*)-Phytol		0.6							1949	2622	Ri, MS
105	Hexadecanoic acid ethyl ester			*t*						1957	2931	Ri, MS, Co-GC
106	Ethyl hexadecanoate		0.1							1994	2245	Ri, MS, Co-GC
107	Heicosane				*t*					2000	2000	Ri, MS, Co-GC
108	(*Z,Z*)-9,12-Octadecadienoic acid methyl ester			0.1						2085	2505	Ri, MS, Co-GC
109	Heneicosane		0.1							2100	2100	Ri, MS, Co-GC
110	Osthole					0.1	*t*	*t*		2138	2449	Ri, MS
111	(*Z,Z*)-9,12-Octadecadienoic acid ethyl ester	0.1								2162	2535	Ri, MS, Co-GC
112	Tricosane		0.2	0.1				0.4		2300	2300	Ri, MS, Co-GC
113	Tetracosane				0.1		*t*	0.1		2400	2400	Ri, MS, Co-GC
114	Pentacosane	0.1	0.2	0.1	0.1		0.2	1.2		2500	2500	Ri, MS, Co-GC
115	Hexacosane	0.1		*t*	*t*					2600	2600	Ri, MS, Co-GC
116	Heptacosane		0.2	0.2	0.2	0.2		2.1	0.1	2700	2700	Ri, MS, Co-GC
117	Octacosane						*t*	*t*		2800	2800	Ri, MS, Co-GC
118	Squalene				0.1	0.1	0.2	0.3	0.1	2829	3048	Ri, MS
119	Nonacosane	0.1	0.1	0.1	0.1	0.1	0.1	2.2	*t*	2900	2900	Ri, MS
120	Triacontane						*t*	*t*		3000	3000	Ri, MS
121	Hentriacontane						0.2	0.7		3100	3100	Ri, MS
	Total amount of compounds	**94.1**	**88.2**	**93.0**	**91.2**	**83.7**	**54.5**	**93.3**	**93.9**	9999		

a) A, aerial parts; F, flowers; R, roots and S, seeds.
b) *t*, trace, less than 0.05%.
c) HP-5 MS column.
d) HP Innowax.
e) RI, Retention index; MS, mass spectrum and Co-GC, co-injection with authentic compound.
f) Isomer not identified.

2.6 Qualitative and quantitative analyses

Most constituents were identified by GC by comparison of their retention indices (*I*) with those of the literature [28, 29] or with those of authentic compounds available in our laboratories. The retention indices were determined in relation to a homologous series of *n*-alkanes (C_8–C_{28}) under the same operating conditions. Further identification was made by comparison of their mass spectra on both columns with those stored in NIST 02 and Wiley 275 Libraries or with mass spectra from the literature [28, 30] and home-made library. Component relative concentrations were calculated based on GC peak areas without using correction factors.

2.7 Scanning electron microscopy

Scanning electron microscopy (SEM) investigation was performed using a Philips XL30 equipped with an Energy Dispersive X-ray device. Samples were supported on the stubs by carbon paint and were coated with gold. The accelerating voltage ranged at 25 kV.

3 Results and discussion

3.1 Composition of essential oil

Starting from different dried parts of *F. campestris*, namely steams and leaves (aerial parts), flowers, roots and seeds, different amounts of essential oils were obtained by means of the different isolation methods. In particular, 0.11, 0.13, 0.05 and 1.50% amounts, respectively, were obtained by MAHD, whereas 0.11, 0.13, 0.10 and 1.05%, respectively, were obtained by HD. MAHD extraction takes 30 min, whereas 3 h were required by HD. At the extraction time of 30 min, MAHD yielded, for aerial parts and flowers, a similar quantity of oil compared with that obtained by HD after 3 h. GC data of essential oils obtained by MAHD and HD are summarized in Table 1, according to their linear retention index (LRI) on an HP-5 MS column and percentage contribution. Totally, 100 compounds were identified in the oils extracted by MAHD; on the contrary, a smaller amount, 88 compounds were detected in the oils extracted by HD. This difference may be due to the loss or the decomposition of chemical components in the course of the steam distillation or a deeper extraction by MAHD than

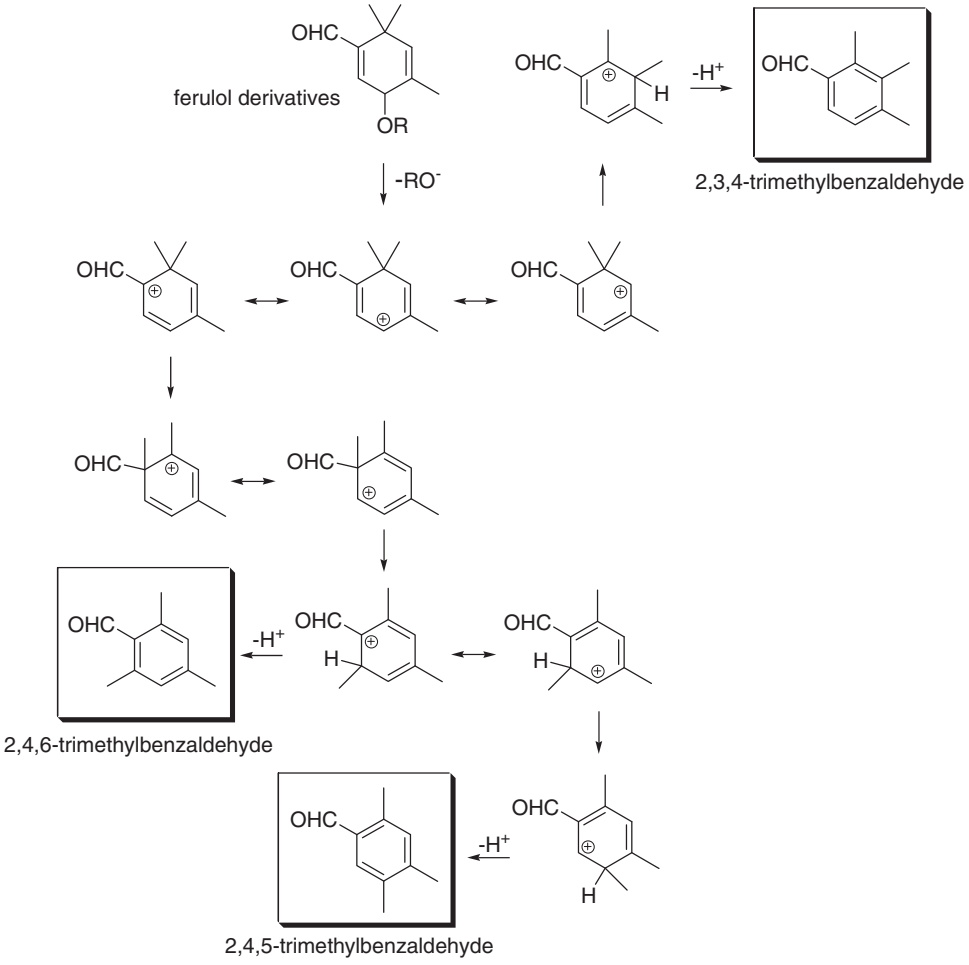

Figure 2. Hypothesized formation of 2,4,5- and 2,4,6-trimethylbenzaldehyde isomers.

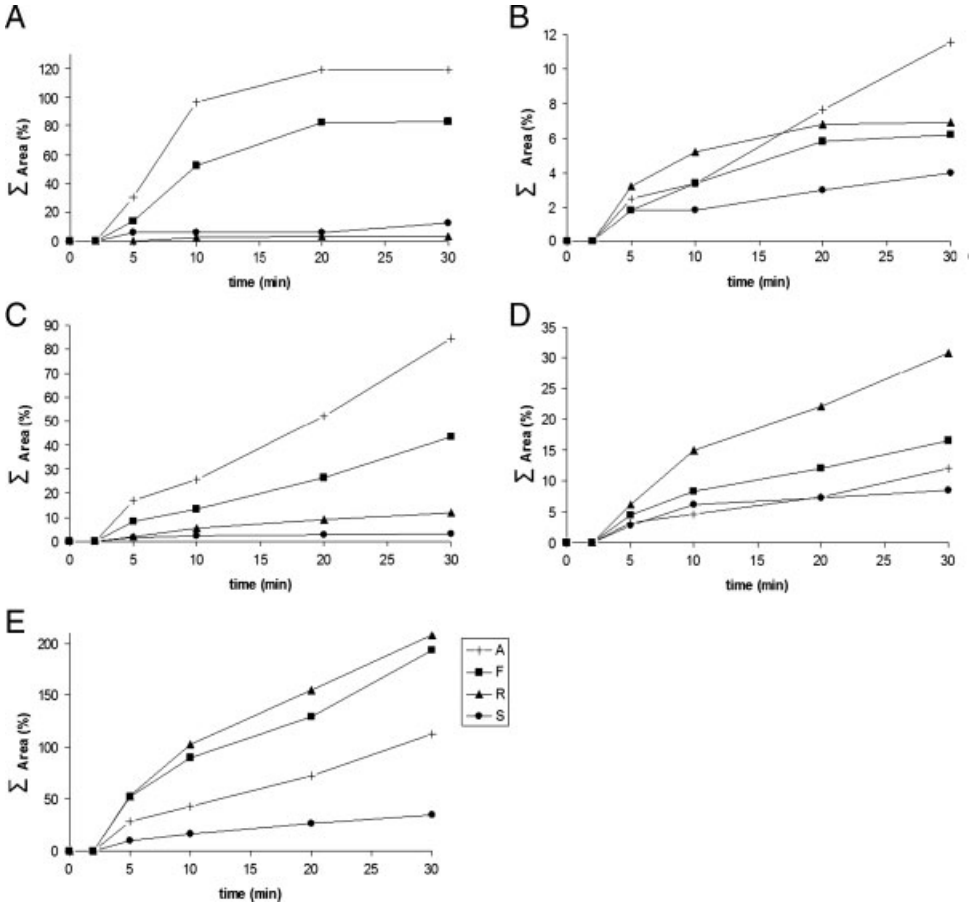

Figure 3. Percentage distribution as function of the time of most representative components, assembled for family, MH (A), OM (B), SH (C), OS (D) and BA (E) in the different parts of *F. campestris* extracted by MAHD method.

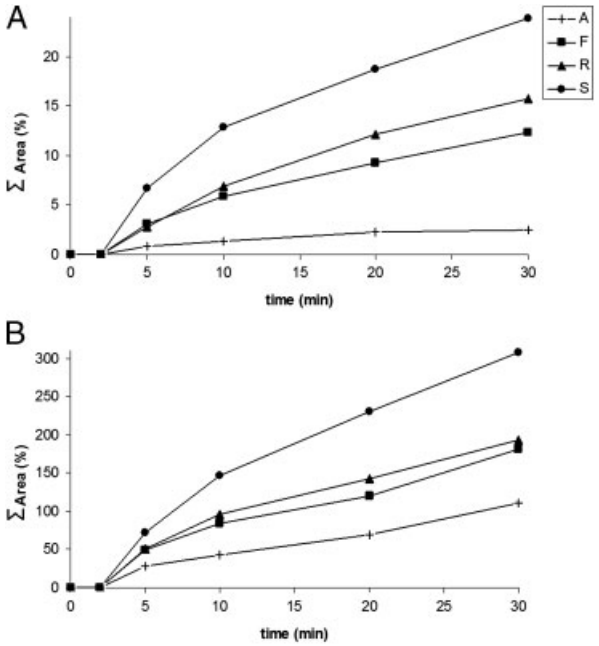

Figure 4. Percentage distribution as function of the time of 2,4,5-trimethylbenzaldhehyde (A) and 2,4,6-trimethylbenzaldhehyde (B) in the different parts of *F. campestris* extracted by MAHD method.

HD [6]. In the oils extracted by MAHD, among 100 compounds, 56 of them were found in aerial parts (A), 52 in the oil from flowers (F), 44 in the oil from root (R) and 39 in that of seeds (S). In the oil extracted by HD, among 88 compounds, 51 of them were found in A, 62 in the oil from F, 44 in the oil from R and 38 in that of S. With the exception of the oil from R, the percentage distribution of compounds was very similar comparing MAHD with HD methods.

The most prominent components found in all different parts of *F. campestris*, and both extraction methods, were two aldehyde isomers (BAs) followed by monoterpene hydrocarbons (MH), sesquiterpene hydrocarbons (SH) and a lower quantities of oxygenated monoterpenes (OM) and oxygenated sesquiterpenes (OS).

As summarized in Table 1, wide diversity in the composition of essential oils, depending on the organ of the plant analyzed, was observed. Furthermore, it was interesting to note that the percentage of the constituents of oils extracted depended on MAHD or HD method adopted. In particular, between the two aldehydes, the 2,4,5-trimethylbenzaldehyde (entry 46, Table 1) was the greatest isomer extracted by HD in A and S, whereas the 2,4,6-trimethylbenzaldehyde (entry 49, Table 1) was the major compound extracted by MAHD in A, F, S and by HD in R.

The presence of 2,3,4- and 2,3,6-trimethylbenzaldehyde, in the essential oils and extracts of *Ferulago* species, was already observed [31, 32]. It is due to the cleavage and subsequent rearrangement of ferulol type monoterpenoids caused by thermal treatment, as previously established by comparing the compositions of essential oils obtained by steam HD and supercritical fluid extraction [32]. It is noteworthy that the presence of essential oils of BA's isomers in the HD and MAHD was not previously observed (2,4,6- and 2,4,5-), and whose occurrence can be explained by sequential rearrangements of a carbocation arising from thermal cleavage of several ferulol derivatives (Fig. 2) that could be in all of the parts of the plant but surely in the flower as reported earlier [33].

The oils extracted by HD were richer in MH and OM in all different parts of plant. Representative MH constituents in both methods were *p*-cymene and γ-terpinene in A, F and S (entries 7 and 11, Table 1), α-pinene in A, F, R and S (entry 1, Table 1), myrcene in A by HD only and F (entry 5, Table 1), pseudocumene in S (entry 4, Table 1). Among the OMs, the *p*-cymen-8-ol was the most representative constituent in A (by HD only), F (by HD only), and R and S (entry 27, Table 1).

SH was the most abundant fraction in A when we used MAHD method, whereas no significant differences of composition were found in the remaining parts of *F. campestris*. Representative SH constituents in both methods were β-caryophyllene, β-selinene and δ-cadinene in A, F, R and S, respectively (entries 58, 69 and 76, Table 1).

Finally, OS, less abundant than hydrocarbon derivatives, appeared in greater percentage by MAHD in A, F and R; an opposite result was observed in S.

Representative OS constituents in both methods were caryophyllene oxide in A, F, R and S (entry 82, Table 1), whereas spathulenol in S only (entry 83, Table 1).

3.2 Kinetic of microwave extraction

It seemed interesting to evaluate how much each class of compounds was extracted as function of time. This analysis was carried out for the MAHD method; we chose this method because it is both faster and easier than the HD in collecting data. Figure 3 shows the quantities of components, assembled for family, extracted at five different times (2, 5, 10, 20 and 30 min). The quantity at each time was evaluated as sum of GC area (Supporting Information, Tables 4–7). In doing this, of course, we were aware about the fact that the relative areas of the GC peaks for the different components do not correspond to the relative composition of the mixture. However, it can be confidently assumed that such a condition is approximatively true as long as single classes of structurally homogeneous compounds are concerned.

Some graphs mentioned above report curves of sigmoidal type, whereas others show curves that can be interpreted as two sigmoidal trends having different induction periods [16]. In our opinion, the presence of two different induction periods should imply that the compound is located in two different cell types. Hence, a short induction period should represent the fraction of essential oil extracted from trichomes, special secretory structures located on the surface of different organs of *F. campestris*, whereas a larger induction period represents an inner location in plant tissues of secretory structure containing the extracted species. This hypothesis seems to be supported by the fact that in some cases all curves present the same induction period irrespective of family chemical–physical properties; in other cases, the different induction periods are not attributable to characteristic of the families. This seems to confirm that, for example, BAs are present in F and S in different secretory structures (Fig. 3E).

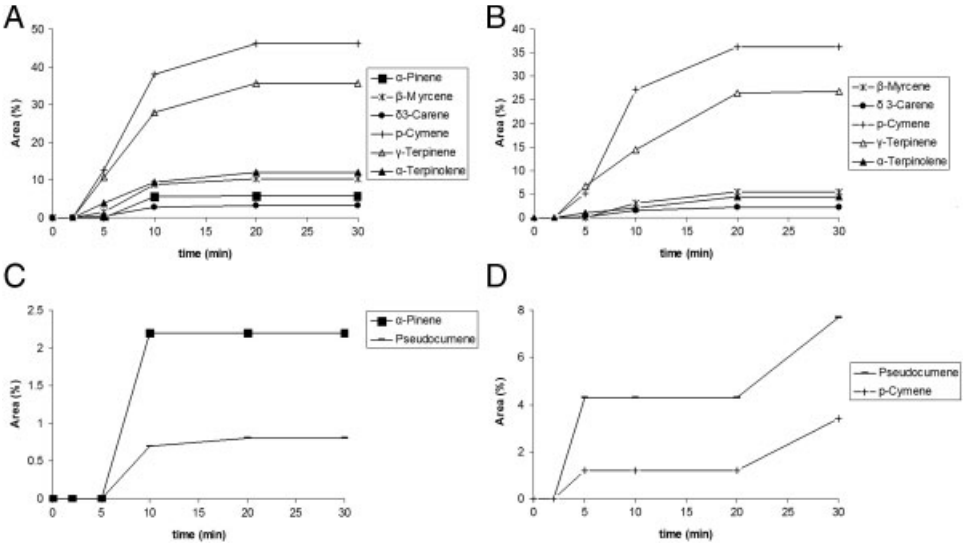

Figure 5. Percentage distribution as function of the time of most representative MH components of essential oil in A (A), F (B), R (C) and S (D) parts of *F. campestris* extracted by MAHD method.

In order to establish how each constituent of a family is extracted, the same analysis was carried out for the most representative components. For BA family, a parallel behaviour in each part of the plant was detected for the two components, and hence, for example, as seen above, in A and F two induction times (2 and 20 min) were observed (Fig. 4B).

For MH family (Fig. 5), the principal components show a sigmoidal trend in A, F and R parts. In S, the curves of two components, pseudocumene and *p*-cymene, have two induction periods (2 and 20 min). In R, a unique induction period was observed (5 min), whereas in A and F two induction periods (2 and 5 min) were observed.

For SH family, a more articulated situation can be detected. The principal components have a sigmoidal trend with different induction periods (2, 5, 10 and 20 min). In Fig. 6, the trend of one representative compound of this family is reported. As shown in the figure, the trend of β-selinene presents one induction period at 2 min in all parts of the plant and a second one at 10 and 20 min in A and F, respectively. Furthermore, at 20 min the quantity of β-selinene in A is greater than in F, and for extraction time greater than 30 min, the quantity of β-selinene is most abundant in F.

Similar results were obtained by the analysis of curves for each component in the other parts of plants.

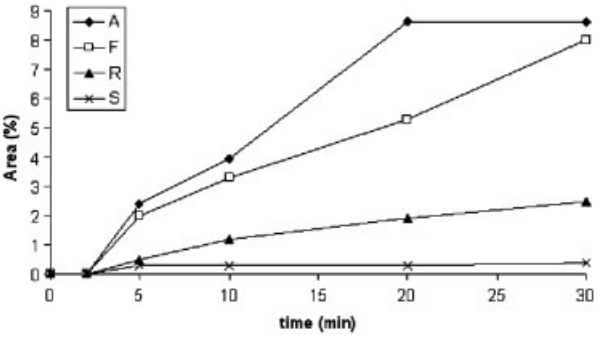

Figure 6. Percentage distribution as function of the time of β-selinene representative SH component of essential oil in A (A), F (B), R (C) and S (D) parts of *F. campestris* extracted by MAHD method.

A further parameter that can be deduced by the above-reported curves is the extraction rate, expressed as percent of extract in the time unit (min). For example, the rate values of some MH components in A (Fig. 5A) result to be 8.2, 7.9 and 7.8 for *p*-cymene, γ-terpinene and terpinolene, respectively. These very similar values could reflect the similar values of boiling points of compounds (177, 183 and 178°C, respectively).

However, rate values of 7.5 and 5.6 were calculated in F (Fig. 5B) for *p*-cymene and γ-terpinene, respectively. Thus, a comparison of rate values in A and F seems to indicate that, in the first case, the components were extracted either by the same cell or by the cells having comparable permeability; instead in the second case, different cells were implicated.

It is interesting to note that extraction values of 8.2, 7.5 and 10.0 were calculated for *p*-cymene in A, F and S, respectively. This could be attributed to the different locations of cells present in different parts of plant.

3.3 Morphological changes of plant material after extraction

SEM investigation of the different parts of *F. campestris* was performed before and after the MAHD and the HD extraction. As an example, same micrographs of the seed surface are shown in Fig. 7.

Figure 7A is a micrograph of the untreated integument that has the task to cloth and protect the seed, whereas Fig. 7B and C is the image of the seed after HD and MAHD extraction, respectively. It can be seen that HD method gave an effect of explosion integument and some cells of the parenchyma reserve now were visible; MAHD extraction instead caused the total removal of the integumental tissue and damaged parenchymal cells.

SEM observation of *F. campestris* seems to confirm that MAHD is an efficient and rapid method of oil extraction causing, on 30 min only, a considerable physical change on system tissue core on all organs of the plant analyzed. Indeed, both constituents of external integumental or xylem tissues, composed of strong lignified cells too, and internal parenchymatous or secretory tissues clearly showed that the cells are broken and damaged during MAHD treatment. On the contrary, in some organs, the changes observed for HD

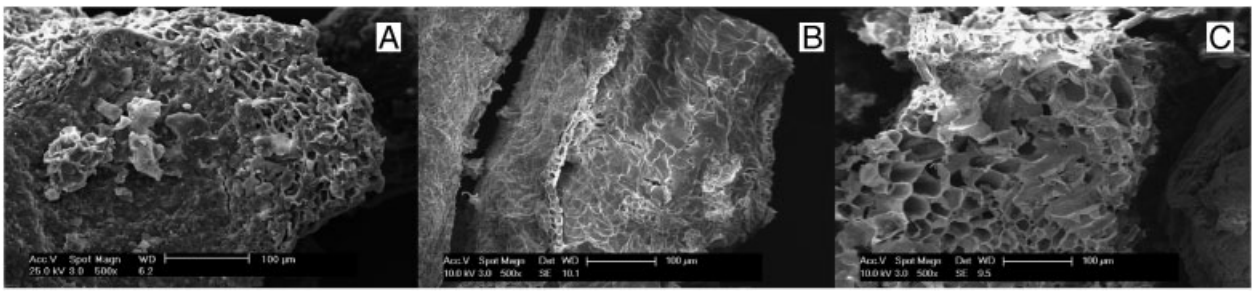

Figure 7. Scanning electron micrographs of *F. campestris* seeds: (A) untreated, (B) after HD for 4 h and (C) after MAHD for 30 min.

extraction method, on 4 h, were less profound with respect to those observed by MAHD on 30 min.

4 Concluding remarks

The collected data show that the used method (MAHD or HD) for the extraction of essential oil from components (aerial parts, flowers, roots and seed) of *F. campestris* influences the composition of oils as well as the ease of extraction.

The SEM analysis of the *F. campestris*, before and after the extraction, showed that MAHD treatment makes deeper morphological changes of plant material than the HD method; this is in accordance to a more efficient extraction.

Moreover, the composition of the oil extract by means of MAHD method at various times and the rate of extraction process for the principal compounds in the various plant components was determined. The results have shown that the facility of extraction depends on location of cell in which a substance is present.

This study was conducted using a self-assembled microwave apparatus. It could be interesting to extend the study in a dedicated batch or to continue microwave apparatus for the scale-up in order to know the effect of the process parameters in the microwave extraction of essential oils.

The authors acknowledge Professor Renato Noto and Professor Paolo Colombo for their precious support. The GC-MS spectra were performed at the "C.S.I.A.S." of the University "Federico II" of Napoli. The assistance of the staff is gratefully appreciated. Thanks are due to MIUR for supporting this research through the PRIN 2007R3PXF 002 and PRIN 2008KRBX3B.

The authors have declared no conflict of interest.

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