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Original Paper

Effects of solvent-free microwave extraction on the chemical composition of essential oil of *Calamintha nepeta* (L.) Savi compared with the conventional production method

The essential oil of *Calamintha nepeta* has been obtained by solvent-free microwave extraction (SFME) and by classical hydrodistillation (HD). A comparative qualitative-quantitative study on the composition of the oils was carried out. A total of 38 compounds, constituting 97.6% of the oil, were identified in the oil obtained by SFME, whereas 46 compounds, representing 95.4% of the oil, were characterized in the HD oil. SFME-distilled oil is richer in lightly oxygenated monoterpenes (LOM) than HD oil. It also has a higher amount of sesquiterpenes and a lower quantity of hydrocarbon monoterpenes. HD oil seems to be affected by chemical changes more than SFME oil.

Keywords: *Calamintha nepeta* / Essential oils / Hydrodistillation / Solvent-free microwave extraction

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1 Introduction

Recently the use of microwave-assisted hydrodistillation (MAHD) [1 – 3], compared to different techniques [4] such as classical hydrodistillation (HD), supercritical fluid extraction (SFE), and organic solvent extraction (SE), has been described as an optimum tool for the extraction of *Rosmarinus officinalis* L. oil [5]. However, a pre-treatment of plant material and relatively high irradiation power, compared to the technique herein reported, were necessary. Furthermore, no significant variations in the quantitative composition of HD and MAHD oils with respect to the ranges reported in Pharmacopoeia have been established. Chemat *et al.* developed a new technique combining microwave heating and dry distillation for isolation of essential oils from fresh plant materials at atmospheric pressure [6]. In several cases the composition of the essential oil proved to be richer in oxygenated compounds on preparation by solvent-free microwave extraction (SFME) than by the HD method [7, 8].

In order to compare the efficacy of SFME and HD methods in extraction processes of natural products we have carried out the essential oil extraction of *Calamintha nepeta*.

Calamintha nepeta (L.) Savi (Syn.: *Satureja calamintha* (L.) Scheele, *Satureja nepeta* (L.) Scheele) is a perennial aromatic plant which belongs to the Lamiaceae family [9]. Numerous members of this family are used as spice, and are also employed in folk medicine in diverse traditions. According to popular knowledge, *C. nepeta* is effective against convulsion and cramps, and as an antiseptic and a diuretic. Modern medicine acknowledges the essential oil of *C. nepeta* to have antimicrobial activity.

In the literature there are many reports about essential oil chemical composition and antimicrobial activity of *Calamintha* species growing in several regions of Europe [10 – 14]. Classic distillation methods have been described and great variability in composition has been observed [12], due both to the diversity of species and to the extraction procedures [14]. *C. nepeta* oil is reported to be rich in highly odorous, lightly oxygenated monoterpenes (LOM) of menthone, pulegone, piperitone, and piperitenone types [11]. Flamini *et al.* reported antimicrobial activity against gram-positive bacteria, in particular for the authors pulegone is the most effective component among the constituents of the essential oil [15].

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Abbreviations: FID, flame ionization detection; **HD**, hydrodistillation; **LOM**, lightly oxygenated monoterpenes; **MAHD**, microwave assisted hydrodistillation method; **SE**, solvent extraction; **SFE**, supercritical fluid extraction; **SFME**, solvent-free microwave extraction

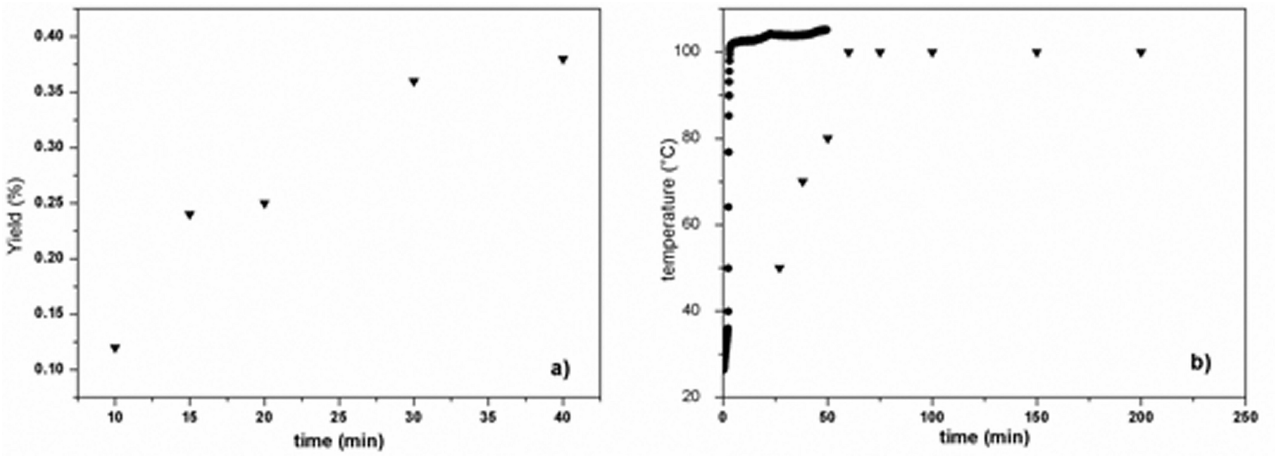

Figure 1. a) Yields as a function of time for SFME. b) Temperature profiles as a function of time for the HD (▼) and SFME (●) extractions of essential oil of *Calamintha nepeta*.

Unfortunately LOM are vulnerable to chemical changes under steam distillation conditions [16]; therefore, it is advisable to use a mild and fast method for the extraction of oil containing a large amount of LOM.

SFME was performed without the use of water, classical HD using a Clevenger-type apparatus. A scanning electron micrograph (SEM) investigation was carried out on the leaves of *C. nepeta* before and after SFME.

2 Experimental

2.1 Plant material

Fresh *Calamintha nepeta* (L.) Savi was collected in Castelbuono (Sicily) in November 2006. It was authenticated by the Botanic Department of University of Palermo with specimen number PAL 382/06.

2.2 Microwave apparatus and procedure

The microwave apparatus was a Sairem downstream microwave source working at 2.45 GHz [17]. The main characteristic of this set-up are: the homogeneous field distribution inside the sample holder; the ability to measure the microwave power absorbed by the sample; the simplicity of use.

Incident (*Pi*), reflected (*Pr*), and transmitted (*Pt*) power were continuously measured by means of power sensors and power meters connected before (by means of a reflectometer bridge) and behind the sample holder, respectively. More details on apparatus and field dosimetry are reported elsewhere [18].

Sample temperature can be continuously monitored during the treatment by a non-perturbative fibre optic thermometer (Nortech ReFlex TP21M02).

In a typical SFME procedure, performed at atmospheric pressure, 60 g of aerial parts of fresh plant material were placed in the glass cylinder, inserted in the waveguide, and irradiated using a fixed incident power of 250 W for 40 min until no more essential oil was obtained and the plant material was dried (see Fig. 1a). The non-perturbative fibre optic thermometer was introduced inside the glass cylinder to monitor the temperature during the treatment. A Clevenger refrigerator provided with a glass stopcock and a circulating water condenser was connected to the sample holder to collect the extracted essential oil. The energy is absorbed by the water causing a temperature increase and it is distributed throughout the system. After 3 min, the temperature reaches 100°C (see Fig. 1b) and the water starts evaporating. The essential oil–water mixture was extracted with *n*-pentane after condensation. The organic phase was evaporated at 35°C under argon at atmospheric pressure and dried over anhydrous sodium sulphate. The essential oil was stored at 4°C until used.

2.3 Hydrodistillation apparatus and procedure

60 g of fresh *Calamintha nepeta* was submitted to hydrodistillation for 4 h with a Clevenger-type apparatus according to the standard procedure described in the European Pharmacopoeia [19], using *n*-pentane as a solvent. The organic phase was evaporated at 35°C under argon at atmospheric pressure and dried over anhydrous sodium sulphate. The essential oil was stored at 4°C until used.

2.4 Gas chromatography

Analytical gas chromatography was carried out on a Perkin-Elmer Sigma 115 gas chromatograph fitted with a HP-5 MS capillary column (30 m × 0.25 mm id; 0.25 μm film thickness). Helium was the carrier gas (1 mL/min). The column temperature was initially kept at 40°C for

5 min, then gradually increased to 250°C at 2°C/min, 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 carried out using a fused silica HP Innowax polyethylene glycol capillary column (50 m × 0.20 mm id; 0.20 μm film thickness).

2.5 Gas chromatography – mass spectrometric 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. Gas chromatographic conditions were as reported above; transfer line temperature, 295°C.

2.6 Qualitative and quantitative analyses

Most constituents were identified by gas chromatography by comparison of their retention indices (*I*) with those of the literature [20, 21] 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_{24}) under the same operating conditions. Identification was confirmed by comparison of mass spectra of components isolated from both columns with those stored in NIST 02 and Wiley 275 Libraries or with mass spectra from literature [20, 22] and our library. Relative concentrations of the component were calculated by GC peak areas without using correction factors.

2.7 Scanning electron micrographs (SEM)

SEM investigation was performed by using a Philips XL30 equipped with an Energy Dispersive X-ray device. Samples were supported on the stubs by carbon paint. The accelerating voltage ranged between 20 and 25 kV.

3 Results and discussion

3.1 Composition of essential oil

The same weight of plant material releases the same amount of essential oil faster (30 min) under microwave action (SFME) than on HD (4 hours). Specifically, 213 mg and 219 mg of essential oil were obtained from 60 g of fresh plant material by SFME and HD, respectively. GC – MS analysis of essential oils of *C. nepeta* (Table 1), obtained by SFME and HD, showed that both are mainly composed of oxygenated monoterpenes (OM) with a smaller quantity of sesquiterpenes. In particular, we could distinguish

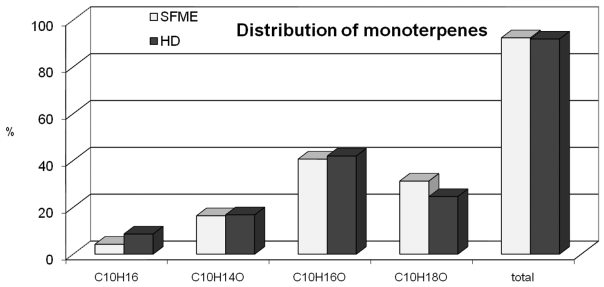

Figure 2. Distribution pattern of the main monoterpene classes in the oils of *Calamintha nepeta* distilled by solvent-free microwave extraction (SFME) and by hydrodistillation (HD).

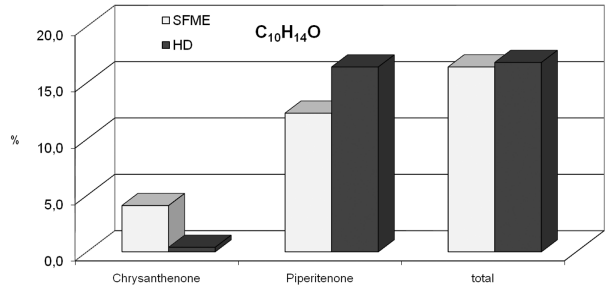

Figure 3. $C_{10}H_{14}O$ monoterpenes in the oils of *Calamintha nepeta* distilled by solvent-free microwave extraction (SFME) and by hydrodistillation (HD).

three main classes of OM, having a $C_{10}H_{14}O$, $C_{10}H_{16}O$, and $C_{10}H_{18}O$ molecular formula, respectively, and one class of hydrocarbon monoterpenes (HM) with $C_{10}H_{16}$ molecular formula. Table 1 also reports the classification of oil components according to these classes. As shown in the table, the major compounds in the oils were pulegone (25.2% and 21.4%, respectively, in oils obtained by SFME and HD) and other ketones with the *p*-menthane skeleton such as piperitone (13.1%, SFME, and 6.4%, HD), piperitenone (12.3%, SFME, and 16.4%, HD), menthone (11.6%, SFME, and 19.8%, HD), isomenthone (12.0%, SFME, and 2.1%, HD). The SFME-distilled oil is composed of 93.7% of monoterpenes and about the same (93.4%) percentage has been found in the HD oil. Moreover SFME-distilled oil had a lower amount of HM (5.2%) than HD-distilled oil (9.5%) whereas the former was richer in $C_{10}H_{18}O$ compounds (31.3% *vs.* 24.7%). Anyhow, limonene was the most representative monoterpene hydrocarbon in both oils. Chemat *et al*., in a multivariate study on SFME-distilled oil of cardamom (*Elletaria cardamomum*) seeds, reported that similar difference in the composition of the oxygenated compounds is probably due to a diminution of thermal and hydrolytic effects in SFME compared with HD [7, 8].

Figure 2 shows the distribution pattern of the component classes for SFME and HD distillation. Both distillation processes showed a similar qualitative composition;

Table 1. Chemical composition and compounds distribution of the essential oil of *Calamintha nepeta* (L.) Savi (Lamiaceae) extracted by solvent-free microwave extraction (SFME) and hydrodistillation (HD).

Ret[a)]	Ret[b)]		SFME (%)[c)]	HD (%)[c)]	Identification[d)]
Hydrocarbon monoterpenes					
		$C_{10}H_{14}$			
1025	1280	*p*-Cymene	0.4	0.3	RI, MS, Co-GC
		$C_{10}H_{16}$			
930	1014	α-Thujene	0.1	0.1	RI, MS
938	1032	α-Pinene	0.6	1.7	RI, MS, Co-GC
973	1132	Sabinene	1.0	1.1	RI, MS
980	1118	β-Pinene	–	0.9	RI, MS, Co-GC
1001	1146	Δ^2-Carene	0.1	0.5	RI, MS, Co-GC
1012	1189	α-Terpinene	0.2	0.7	RI, MS, Co-GC
953	1076	Camphene	T	T	RI, MS, Co-GC
1038	1245	(*Z*)-β-Ocimene	0.1	0.1	RI, MS, Co-GC
1129	1386	*allo*-Ocimene[n.i.]	T	–	RI, MS, Co-GC
993	1174	Myrcene	0.4	0.4	RI, MS, Co-GC
1030	1203	Limonene	2.1	2.8	RI, MS, Co-GC
1057	1256	γ-Terpinene	0.2	0.9	RI, MS, Co-GC
		Total $C_{10}H_{16}$	4.8	9.2	
Oxygenated monoterpenes					
		$C_{10}H_{14}O$			
1125	1540	Chrysanthenone	4.1	0.4	RI, MS
1329	1949	Piperitenone	12.3	16.4	RI, MS
		Total $C_{10}H_{14}O$	16.4	16.8	
		$C_{10}H_{14}O_2$			
1363		Piperitenone oxide	0.1	0.2	RI, MS
		$C_{10}H_{16}O$			
1175	1582	Isopulegone[n.i.]	2.3	14.1	RI, MS
1233	1662	Pulegone	25.2	21.4	RI, MS, Co-GC
1343	1748	Piperitone	13.1	6.4	RI, MS, Co-GC
		Total $C_{10}H_{16}O$	40.6	41.9	
		$C_{10}H_{18}O$			
1063	1555	*cis*-Sabinene hydrate	5.7	0.3	RI, MS, Co-GC
1034	1213	1,8-Cineole	T	T	RI, MS, Co-GC
1177	1755	Dihydrocarveol	0.1	0.1	RI, MS
1093	1474	*trans*-Sabinene hydrate	–	0.7	RI, MS
1138	1475	Menthone	11.6	19.8	RI, MS, Co-GC
1163	1502	Isomenthone	12.0	2.1	RI, MS
1176	1611	Terpinen-4-ol	1.7	1.4	RI, MS, Co-GC
1189	1706	α-Terpineol	0.2	0.3	RI, MS, Co-GC
		Total $C_{10}H_{18}O$	31.3	24.7	
		$C_{10}H_{20}O$			
1182	1652	Menthol	T	0.2	RI, MS, Co-GC

Table 1. Continued

Ret[a)]	Ret[b)]		SFME (%)[c)]	HD (%)[c)]	Identification[d)]
		$C_{12}H_{20}O_2$			
1290	1597	Bornyl acetate	0.1	0.1	RI, MS, Co-GC
		$C_{12}H_{22}O_2$			
1294		Menthyl acetate	T	–	RI, MS
Hydrocarbon sesquiterpenes					
1377	1497	α-Copaene	0.1	–	RI, MS
1378	1600	β-Elemene	0.1	–	RI, MS
1382	1547	β-Cubebene	T	–	RI, MS
1385	1535	β-Bourbonene	0.2	0.1	RI, MS
1455	1689	α-Humulene	0.1	0.1	RI, MS
1415	1612	Caryophyllene	1.2	0.9	RI, MS, Co-GC
1477	1726	Germacrene D	0.7	T	RI, MS
1515	1776	γ-Cadinene	–	T	RI, MS
1520	1760	*epi*-Sesquiphellandrene	0.1	T	RI, MS
1526	1173	δ-Cadinene	–	T	RI, MS
		Total	2.5	1.1	
Oxygenated sesquiterpenes					
1580	2008	Caryophyllene oxide	0.3	0.2	RI, MS, Co-GC
1640	2187	T-Cadinol	–	T	RI, MS
1649	2255	α-Cadinol	–	T	RI, MS
1642	2209	T-Muurolol	–	T	RI, MS
		Total	0.3	0.2	
Others					
992	1394	Octan-3-ol	1.1	0.5	RI, MS, Co-GC
1353	2186	Eugenol	–	0.1	RI, MS
1451	1868	Geranyl acetone	–	T	RI, MS
1835	2131	Hexahydrofarnesylacetone	–	0,1	RI, MS
2800	2800	Octacosane	–	T	RI, MS
1950	2622	Phytol	–	T	RI, MS
		Total	1.1	0.7	
		Total components	97.6	95.4	

a) HP-5 MS column.
b) HP Innowax.
c) T = trace, less than 0.05%.
d) RI is the retention index, MS = mass spectrum, Co-GC = co-injection with authentic compound. n.i. = isomer not identified.

nevertheless, the profile of distribution of monoterpenes is quite different for the two methods used.

Both essential oils comprised about 17% $C_{10}H_{14}O$ monoterpenes but their composition is different (Fig. 3). In fact, 4.1% and 0.4% of chrysanthenone have been found in oil obtained by SFME and HD methods, respectively, whereas 12.3% and 16.4% of piperitenone were recovered by SFME and HD, respectively. These data seem to indicate that HD promoted the conversion of chrysanthenone into piperitone (Fig. 4a). An alternative explanation, *i.e.* conversion of piperitenone into chrysanthenone by the effect of MW, seems unlikely because the α,β-unsaturated piperitenone is seen to be more stable than the strained four-membered ring chrysanthenone.

The main class of monoterpenes occurring in *C. nepeta* oils, representing about the 40% of composition, was constituted of $C_{10}H_{16}O$ products, also having a different distribution (Fig. 5). In particular, 2.3% and 14.1% of isopule-

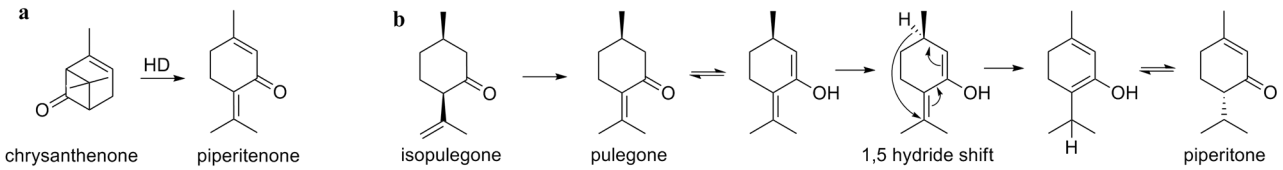

Figure 4. a) Conversion of chrysanthenone into piperitenone; b) hypothesized transformation sequence of isopulegone into piperitone.

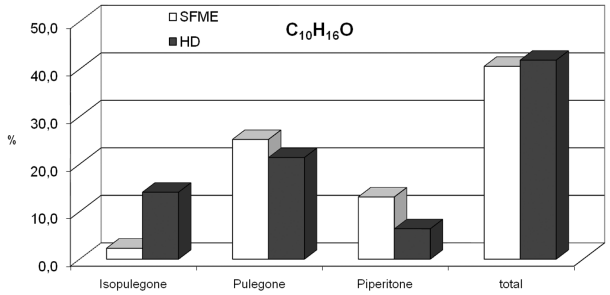

Figure 5. $C_{10}H_{16}O$ monoterpenes in the oils of *Calamintha nepeta* distilled by solvent-free microwave extraction (SFME) and by hydrodistillation (HD).

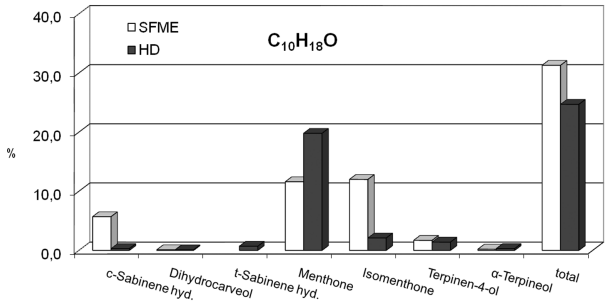

Figure 6. $C_{10}H_{18}O$ monoterpenes in the oils of *Calamintha nepeta* distilled by solvent-free microwave extraction (SFME) and by hydrodistillation (HD).

gone, 25.2% and 21.4% of pulegone, 13.1% and 6.4% of piperitone were obtained by SFME and HD methods, respectively. The above compounds can be linked through the reaction sequence depicted in Fig. 4b. Probably the isopulegone conversion into more stable α,β-unsaturated pulegone is induced by MW.

Interestingly, the composition of $C_{10}H_{18}O$ monoterpenes (Fig. 6) showed the major difference between the two methods. HD distillation gave a lower amount of isomenthone and a higher amount of menthone than SFMW. It is possible that HD favours isomerization because of the prolonged water-contact time at high temperature and decrease of pH during hydrodistillation [23]. The ratio isomenthone/menthone (0.11) found for HD favours the more stable product, indicating the occurrence of suitable conditions for a keto-enolic equilibrium (Fig. 7a).

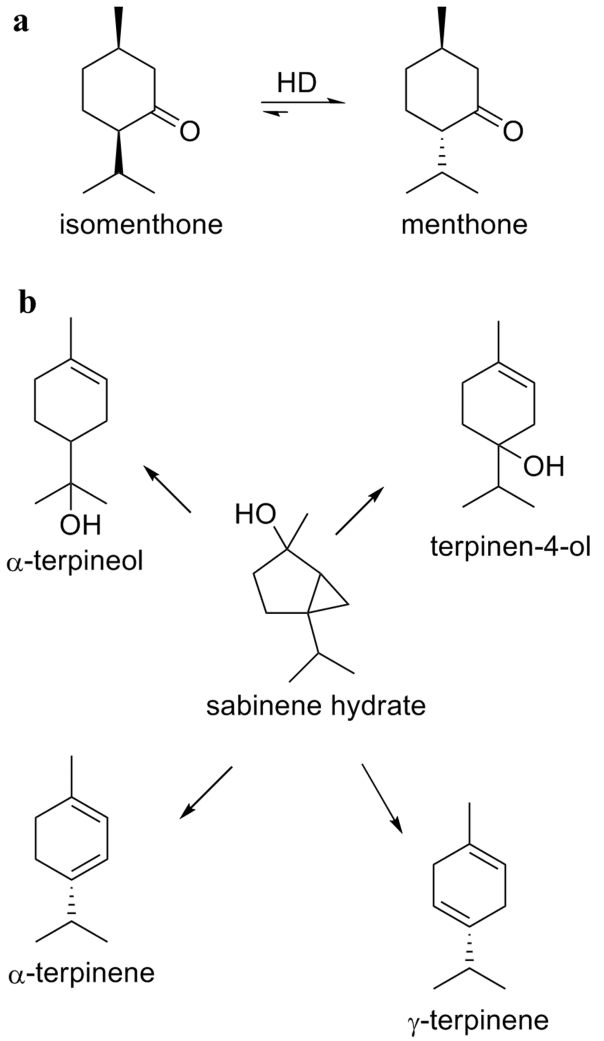

Figure 7. a) Thermodynamic equilibrium isomenthone-menthone. b) Thermal rearrangement of sabinene hydrate.

Furthermore, SFMW distillation gives a higher content of thermolabile sabinene hydrate. *In vitro* sabinene hydrate synthase generally produces both *trans* and *cis* isomers in the fixed ratio of 1:10 respectively. *In vivo* this ratio can be higher in some cases [24]. This ratio has been found about 2:1 in the HD oil, indicating that some rearrangement of *cis*-sabinene hydrate occurs.

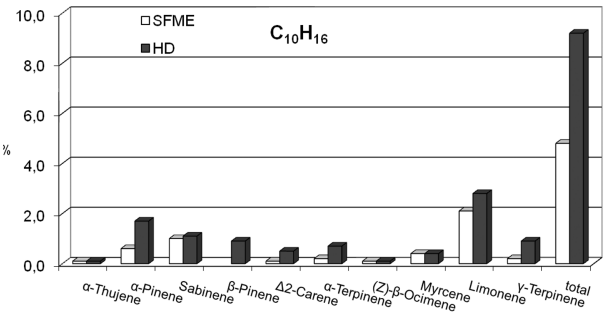

Figure 8. $C_{10}H_{16}$ monoterpenes in the oils of *Calamintha nepeta* distilled by solvent-free microwave extraction (SFME) and by hydrodistillation (HD).

These findings could be explained evoking the previously known thermal rearrangement of sabinene hydrates giving terpinen-4-ol, α-terpinol, α-terpinene, and γ-terpinene (Fig. 7b) [25]. In the hydrocarbon monoterpenes composition for HD (Fig. 8), dehydrated products of sabinene hydrates appeared in higher amounts than SFME. They are mainly α-terpinene and γ-terpinene but there are also α-pinene and β-pinene, whose origin is not established, probably originating from $C_{10}H_{18}O$ products.

Furthermore a higher amount of both hydrocarbon and oxygenated sesquiterpenes has been recovered by SFME distillation than by classical HD.

3.2 Morphological changes of plant material after extraction

SFME produced distinguishable physical changes in the aerial parts of *C. nepeta* Figure 9, an SEM micrograph of the untreated C. *nepeta*, can be compared with structures of the treated plant material. Microwaves interact selectively with polar molecules present in glands, trichomes, or vascular tissues. Localised heating leads to cell rupture.

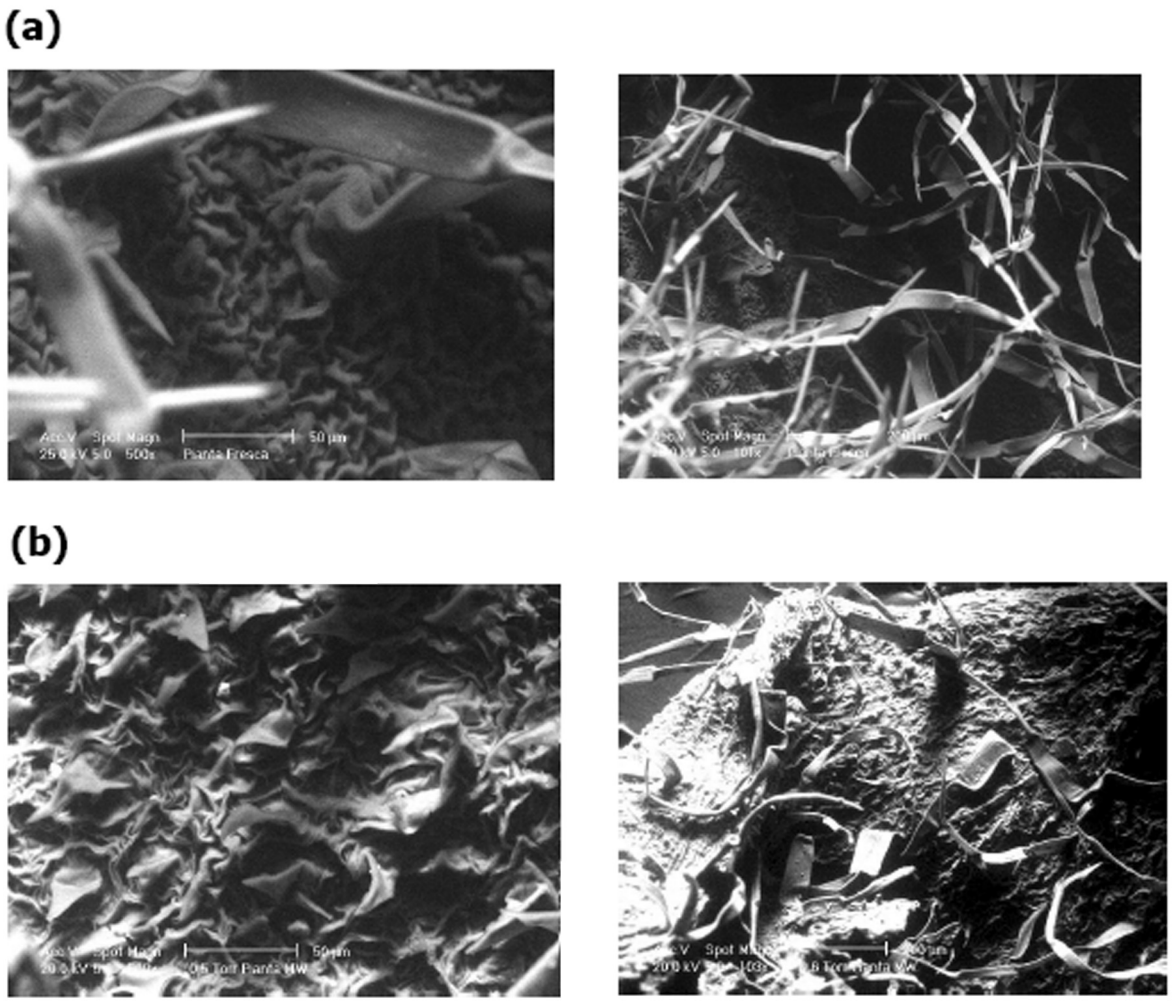

Figure 9. SEM micrographs of *Calamintha nepeta*: a) untreated; b) after SFME extraction (30 min).

4 Concluding remarks

Use of SFME to obtain the essential oil of *Calamintha nepeta* seems to cause fewer chemical changes of the original plant components, *i.e.* less rearrangement, dehydration, and isomerization processes occur than in the case of HD. Furthermore, higher amounts of oxygenated monoterpenes and sesquiterpenes have been recovered from *C. nepeta* by SFME. Lightly oxygenated monoterpenes are the main contributors to aroma of many essential oils; therefore SFME can be a suitable method for producing an oil with more aromatic properties with a simple apparatus less expensive than SFE.

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