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Hydrodistillation and *in situ* microwave-generated hydrodistillation of fresh and dried mint leaves: a comparison study

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Abstract

BACKGROUND: Hydrodistillation (HD) has been used since ancient times for the extraction of essential oils (EO). Despite the intrinsic limitations of this technique, it remains the most common method both in the lab and on an industrial scale. The main drawbacks are the long extraction time involved and the risk of thermal degradation. Over the last decade, microwave-assisted extraction (MAE) and *in situ* microwave-generated hydrodistillation (MGH) have been shown to be the most promising techniques in improving plant extraction and hydrodistillation.

RESULTS: In this piece of work we compare HD with MGH in the extraction of several mint species cultivated in Piedmont: *Mentha spicata* L. var. *rubra*, *Mentha spicata* L. var. *viridis* and *Mentha x piperita* L.. MGH requires either fresh plant or rehydrated material, it is extremely fast and allows a reduction in energy consumption and overall cost. All the EO have been analyzed by GC-MS. A mechanism of microwave-generated essential oil extraction has been

proposed to explain the differences in the composition of the oil obtained from this environmentally friendly technique.

CONCLUSIONS: The yields and composition percentages of the EO obtained by HD and *in situ* MGH of fresh and dried mint leaves lie in a relatively narrow range, although MGH is faster. MW polarization effects and the water solubility of the components influences extract composition.

Keywords: *in situ* microwave-generated hydrodistillation, hydrodistillation, mint, essential oils, GC-MS.

INTRODUCTION

Hydrodistillation (HD) is a classic procedure which has been commonly used for the industrial extraction of essential oils (EO) since ancient times.¹ Traditionally the raw plant material is put into a distillation apparatus over heated water and EO are carried out with the flow as the steam passes through the plant material, breaking down the leaf structures. The condensed mix of water and EO is then collected in the receiving vessel and the oil sits on top. After extraction, the EO is often redistilled, to obtain a purer product. HD is a time-consuming process that usually gives low yields.² Over the last few decades different solvents, pressures and heating sources have been employed to shorten extraction times and improve yield and quality. Several techniques, such as supercritical fluid extraction (SFE),³ ultrasound-assisted extractions (UAE)^{4,5} and microwave-assisted extraction (MAE)⁶ have been proposed.⁷ Microwave irradiation (MW) is applied at different stages; during solvent extraction (microwave-assisted solvent extraction, MASE),⁸ directly, to heat up a solvent to aid HD (microwave-assisted HD)⁹ for direct solvent-free extraction (solvent-free microwave extraction, SFME),^{10,11} or combined with ultrasound extraction (US).¹² The latest innovative

technique is microwave-assisted hydrodiffusion and gravity (MHG),¹³ which allows the EO to be collected for it to simply drop, thanks to the force of earth's gravity, out of the MW reactor into the collector flask.

The most popular essential oils are those obtained from mint species which are widely used for flavouring, cosmetic and pharmaceutical preparations. The genus *Mentha* includes 25 to 30 species that grow in the temperate regions of Eurasia, Australia and South Africa.

We have compared traditional HD with *in situ* MGH for the extraction of three mint species cultivated in Piedmont (*Mentha spicata* L. var. *rubra*, *Mentha spicata* L. var. *viridis* and *Mentha x piperita* L.), with the aim of improving the extraction yields, reducing the time needed to collect the oils, obtaining a reduction in costs and energy and avoiding the problems of compound thermal degradation.

MATERIAL AND METHODS

Plant materials

All the fresh and dried plant materials were provided by the experimental centre at the Faculty of Agriculture (Grugliasco, TO, Italy). The studied species were from a third year crop, planted in the field in spring 2009 from rooty turiones of the same mother plants per species cultivated uniformly in the area in previous experiments.¹⁴ The plants were grown in the same environment, in the experimental centre (44°53'11.67''N; 7°41'7.00''E–231 m a.s.l., Carmagnola, TO, Italy), and soil (sandy-loam, according to the USDA criteria). Plant density for each species was ca. 23 plant/m² and plants were grown according to the standard cultural practices used for *M*. × *piperita* L. in the local area. Harvesting took place on 28 June 2011. Leaves were separated from stems and fresh samples went directly to oil extraction, while dried samples were obtained through an oven-drying process, as set out in previous experiments by some of the authors.^{14,15} The EO content was

calculated as the ratio in weight $(^{w}/_{w})$ between the cold oil collected from the burette and the original weight of the material.

HD

Essential oil extraction via the hydrodistillation technique was carried out according to Tibaldi et al.,^{15,16} with slight modifications. The hydrodistillation equipment was assembled by Exacta+Optech (San Prospero, MO, Italy) and was composed of an electrical heating mantle (500 Watts - Thermo Scientific Electro Thermal, Whaltam, MA, USA), a 2 L Pyrex® glass balloon filled with 1.5 L deionized water with a 4 L modified Pyrex® glass balloon filled with the plant material above it, a condenser and a graduated burette to collect the oil.

Method: an aliquot of 300 g of fresh or dried leaves was placed in the steam-distillation system. Each distillation lasted ca. 105 min, which included 45 min in which the leaves were steamed by the boiling water in the glass balloon. The 45 min distillation time started when the first drop of liquid, condensed in the cooling column, dropped into the graduated burette.

MGH

MGH was performed in a NEOS-GR MW oven (Milestone srl, Sorisole, BG, Italy). Dedicated equipment with specific software equipped with videocamera and IR-pyromether was used. The plant was placed in a 4 L beaker, covered with a polypropylene lid with a central hole connected via a glass tube to the external condenser. The latter was connected to a chiller with tubing in which cooling fluid, maintained at around 5-6°C, circulated. Figure 1 shows both the MGH and the HD apparatuses.

Method: an aliquot of 300 g fresh or dried leaves was placed in a 4 L Pyrex® glass beaker and put in the MW oven. Dried leaves were previously rewetted under slight steam flow by mean of a commercial domestic device "*Vaporetto* Polti - Italy" equipped with a flow regulator (calculated swelling: 2 mL/g). Each distillation lasted ca. 45 min, which included 20 in which the leaves were steamed by the internal water content of the plant. The 20 min distillation time started when the first drop of liquid, condensed in the cooling column, dropped into the graduated burette.

Analytical apparatus

All the GC-MS spectra were recorded on an Agilent 6890 gas chromatograph (Agilent Technologies-USA), fitted with a MS Agilent Network 5973 which uses a 30 m long capillary column HP 5-MS (5% phenyl methyl siloxane, i.d. 0.25 mm, film thickness 0.25 µm), a 1:20 split ratio, a 250 °C injector temperature and helium as the carrier gas (1.2 mL/min flow). The GC parameters were set up as follows; initial temp. 50 °C, rate 3 °C/min, final temp. 80 °C, rate 10 °C/min, final temp. 300 °C, held for 10 min. The MS parameters were as follows; low mass 40, high mass 800, MS quad 150 °C, MS source 230 °C. GC-MS enables fast analysis, good separations and reliable quantitative and qualitative data.¹⁷ The samples were prepared by dissolving 0.04 mL of essential oil in 1 mL of chloroform. The identification of products was achieved using the NIST 05 library (National Institute of Standards and Technology).

RESULTS AND DISCUSSION

Essential oils composition

Aim of our work was a comparison of MGH and conventional HD performed simultaneously on fresh and dried mint leaves. As reported in recent literature surveys,¹⁸ GC-MS can be considered the method of choice for the analysis of volatile fractions.

EO yields are reported in Table 1 where it is possible to see that no striking differences in oil yield exist between the different techniques. An extraction time of 20 min with MGH provides yields comparable to those obtained after 45 min by means of HD, which is the reference method in EO extraction. A comparable profile of volatile secondary metabolites was found in the EO isolated by MGH and HD. Carvone, an oxygenated monoterpene, is the main component in the EO extracted from *M. spicata* L. var. *rubra* leaves with both techniques. The recovered amount was 65.50% and

60.27%, for MGH and HD respectively, using fresh leaves, and 71.14% and 60.17% using dried leaves (Table 2). Limonene, a monoterpene hydrocarbon, is present at 5.87% and 6.75% in fresh leaves and at 9.18% and 8.67% in dried leaves, respectively. The third most abundant compound is β -caryophyllene, yielding 3.64% and 4.48% when distilled from fresh leaves while 2.46% and 4.23% when distilled from dried leaves.

Similar compounds are present as major metabolites of M. *spicata* L. var. *viridis* leaves (Table 3). In fresh leaf EO, the most abundant compound is again carvone, with 37.94% and 48.21% and 38.31% and 58.49% in dried leaf EO, when using MGH and HD respectively. In fresh leaf EO, limonene is the second most abundant compound giving 15.83% and 14.21%. β -caryophyllene amounts are inverted with respect to M. *spicata* L. var. *rubra*, with 4.66% for MGH and 3.44% for HD, however 1,8-cineole is the third major compound here, yielding 10.99% and 10.66% for MGH and HD, respectively. In dried leaf EO, carvone is still the most abundant compound, with 38.31% and 58.49% for MGH and HD, respectively, then things differ. β -caryophyllene (9.34%) and β -cubenene (8.82%) are the other two major compounds for MGH (limonene and 1,8-cineole yielding 7.80% and 7.86%, respectively), while 1,8 cineole (7.67%) and limonene (6.39%) are the second and third major compounds obtained when using HD.

Finally, *M. x piperita* L. fresh leaf EO contain the same three major compounds present in the following equivalent relative amounts after MGH and HD extraction (Table 4): isomenthone (27.68% and 31.92%), menthol (24.82% and 22.00%) and menthofuran (16.35% and 16.39%). Dried leaf EO is similar, but shows some differences. Using MGH, menthol (45.98%) is followed by menthone (14.12%) and 1,8-cineole (8.33%), while using HD, menthol (29.46%) remains the first major compound, then followed by isomenthone (28.82%) and menthone (10.71%).

The differences between MGH and HD extraction techniques can be summarized as follows; a higher total amount of oxygenated compounds is present when using MW, while some other degradation products are present in lower amounts and are sometimes completely missing. This can be explained when one considers that MW reduces the extraction time, allowing faster extraction

with a lower amount of water. This leads to minimum degradation in compounds by hydrolysis, transesterification or oxidation, while some oxidation reactions are promoted by the MW irradiation, resulting in a rise of oxygenated compounds. It is worth noting that oxygenated compounds are highly odoriferous and, hence, the most valuable components in the EO. Instead, monoterpene hydrocarbons, which are present in, on average, a lower amount in the MGH extract, are less valuable due to their limited contribution to the fragrance of the EO.

Cost, energy and environmental impact

MGH is more advantageous than HD in terms of costs and energy. The calculated energies required for the different methods are 1.5 kWh for HD and 0.25 kWh for MGH.¹⁹

In developed countries the biggest cause of greenhouse gases are both transportation and electricity production. The carbon footprint is the total amount of greenhouse gases emissions, assessed in carbon dioxide units (tonnes of CO_2 equivalent/year), caused directly and indirectly by a specific manufacturing process. In our case, the calculated carbon footprint is 1200 g CO_2 for HD and 200 g CO_2 for MGH.¹⁹ According to the literature, 800 g of CO_2 are released in the atmosphere to obtain 1 kWh from the combustion of coal or fossil fuel.²⁰

Proposed mechanism

The importance of terpene solubility in water during distillation was first mentioned by Von Rechenberg²¹ in 1910; "the compounds vaporize according to their degree of solubility in the distillation water rather than following the order of their boiling point". In 1982, Koedam²² demonstrated Von Rechenberg's theory by recording the relative amount of limonene (bp 175 °C) and carvone (bp 231°C) distilled as a function of time. It was observed that carvone distils first, despite its higher boiling point, and then decreases in proportion distilled as the proportion of limonene (175°C) increases. In summary, after one hour of distillation, carvone is the major

compound comprising close to 90% of the extract whereas after 16 hours the content of carvone has decreased to around 60%, while limonene content increases from 10% to 40%.

Similar results have been found for the MGH of essential oils., The compounds with the highest boiling point are largely predominant after only 20 mins for *Mentha spicata* L.. In contrast, it can be seen for HD after 45 min of distillation, that the difference in concentration between compounds is definitely less significant than in MGH and sometimes the differences are completely reversed. Carvone is the most abundant component of the MW extract (65.50%) and limonene the second (5.87%), whereas the HD essential oil contains less carvone (60.27%) and more limonene (6.75%). It seems that the phenomenon called "hydrodiffusion" and the mechanism described by Von Rechenberg is more pronounced in the case of MGH (Table 5).

Another explanation may be found in the dielectric properties of EO compounds and their interaction, as a material, with electromagnetic waves. Essential oils are composed of a variety of compounds divided into two main groups; hydrocarbons and oxygenated compounds. For garden mint, the essential oil is principally made up of carvone (oxygenated monoterpene) and limonene (monoterpene hydrocarbon). How does the MW energy effect differ for these two different aromatic compounds? It would be reasonable to believe that for the more polar compounds, the more readily the MW irradiation is absorbed, the better the interaction between the electromagnetic wave and matter is established, and the more the more polar aromatic component contents are obtained. This would appear to correspond well to what has ben observed in the case of carvone (polar compound) versus limonene (non polar compound) (Table 5).

Another explanation and mechanism could be found in electromagnetic interactions; EO contain organic compounds that strongly absorb MW energy. Compounds with high and low dipolar moments could be extracted in various proportions by microwave extraction. Organic compounds that have a high dipolar moment will interact more vigorously with MWs and can be extracted more easily in contrast with aromatic compounds which have low dipolar moments.

CONCLUSIONS

In this work we have shown how the extraction technique influences both essential oil quantity and quality. The extraction process is strongly accelerated by MW irradiation and the quality of the isolated oil is comparable. MGH requires either a fresh plant or rehydrated material, it is extremely fast and allows a reduction in energy consumption and the overall cost to be achieved. We have showed that the differences in oil composition obtained, with this environmentally friendly technique, are related to the water solubility of the components. The reduction in costs and CO₂ emissions makes the scale-up of this technique extremely appealing. True understanding, on the molecular scale, of MW extraction has not yet reached the degree of maturity of other topics such MW synthesis. MW water extraction-hydrodistillation may occur via one of these two proposed extraction mechanisms "hydrodiffusion" or "MW polarisation" or as a combination of both. The two mechanisms are related to the structure of the extracted compounds and their polarity. MW extraction interferes with polarization effects that cannot be easily separated from the physical and chemical properties of extracted molecules.

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Figure 1. MGH and HD apparatuses.



	Fresh leaves and stems		Dried leaves	
	MGH ^a (%)	HD ^b (%)	MGH ^a (%)	HD ^b (%)
Mentha spicata L. var. rubra	0.10	0.08	0.42	0.41
Mentha spicata L. var. viridis	0.31	0.32	0.44	0.45
Mentha x piperita L.	0.49	0.53	0.89	0.91

1 1	Fresh leaves		Dried leaves	
	MGH	HD	MGH	HD
α-Pinene	0.28	0.40	0.62	0.74
Sabinene	0.32	0.46	0.52	0.63
β-Pinene	0.56	0.72	1.02	1.08
2-Thujene	1.16	1.59	1.36	1.47
3-Octanol	0.26		0.24	0.26
Limonene	5.87	6.75	9.18	8.67
1,8-Cineole	2.88	2.48	3.49	3.45
(E)-β-Ocimene	0.48	0.63	0.45	0.52
(Z)-β-Ocimene	0.30	0.34		0.20
γ-Terpinene	0.34	0.36	0.20	0.11
3-Carene		0.81	0.39	1.11
<i>p</i> -Menth-1-en-8-ol			0.12	0.14
(-)-4-Terpineol	0.89	0.71	0.41	0.26
t-Dihydrocarvone	4.29	1.89	1.44	1.73
<i>c</i> -Carveol	0.34	0.71	0.22	0.33
<i>t</i> -Carveol			0.14	0.23
D-Carvone	65.50	60.27	71.14	60.17
Piperitone	0.68	0.49	0.67	0.82
<i>t</i> -Carvone oxide	0.40	0.28	0.27	0.40
<i>c</i> -Carvone oxide	0.48			0.42
Dihydroedulan II			0.07	0.10
Dihydroedulan I	0.15			0.15
Isolimonene		0.58	0.20	
Dihydrocarvyl acetate	1.87			0.32
<i>t</i> -Carveyl acetate	0.34		0.11	0.18
β-Bourbonene	2.87	2.97	1.37	2.47
β-Elemene	0.22	0.42	0.25	0.55
Isocaryophyllene	0.18			0.11
β-Caryophyllene	3.64	4.48	2.46	4.23
α-Caryophyllene	0.29	0.39	0.19	0.38
(+)-Epi-bicyclosesquiphellandrene	0.79	0.98	0.39	0.90
Germacrene D	0.82	1.73	0.76	1.61
Bicyclogermacrene	0.18	0.44	0.14	0.35
α-Muurolene	0.10			0.13
(1S)- <i>c</i> -Calamenene	0.48	0.93	0.27	0.56
Caryophyllene oxide	0.16	1.68	0.11	0.30
1,4-Cadinadiene	0.18	0.59		0.22
τ-Muurolol		0.41		0.12
Total	97.31	94.50	98.20	95.40

 Table 2. Main components of M. spicata L. var. rubra EO yield (%)

Table 3. Main componei	onents of <i>M. spicata</i> L. var. <i>viridis</i> EO yield (%)			
	Fres	h leaves	Drie	ed leaves
	MGH	HD	MGH	HD
α-Pinene	0.82	0.73		
Sabinene	0.92	0.82		
β-Pinene	1.62	1.24	0.63	0.53
2-Thujene	1.02			
3-Octanol	1.09	1.05		
Limonene	15.83	14.21	7.80	6.39
1,8-Cineole	10.99	10.66	7.86	7.67
(E)-β-Ocimene	0.68	0.58		
γ-Terpinene	0.51	0.57	0.37	0.55
c-Sabinene hydrate	1.19	0.76	1.30	0.93
t-Sabinene hydrate	1.17			
Thujone	0.38			
D-Camphor	0.46			
Borneol	2.38	0.21	0.33	
δ-Terpineol		0.43	0.32	
(-)-4-Terpineol	1.11	1.38		1.03
p-Menth-1-en-8-ol	0.42	0.46	0.42	0.57
Dihydrocarveol	1.89	1.63	0.44	1.30
t-Carveol	1.28	1.55	0.53	0.75
Pulegone	0.43	0.52	0.60	0.62
D-Carvone	37.94	48.21	38.31	58.49
Bornyl acetate	0.24		0.76	
Dihydrocarvyl acetate	0.32	0.29	0.42	
t-Carveyl acetate	0.15	0.17	0.24	
β-Bourbonene	2.52	2.21	5.26	3.20
β-Elemene	0.32	0.34	1.34	0.78
<i>c</i> -Jasmone	0.22	0.33	0.40	0.42
β-Caryophyllene	4.66	3.44	9.34	5.61
β-Cubenene		0.32	0.65	0.42
Germacrene D	0.40		1.01	0.59
α-Caryophyllene	0.79	0.21	0.62	
γ-Muurolene	0.24	0.26		0.28
β-Cubebene	2.93	3.18	8.82	5.37
Bicyclogermacrene	0.98	1.06	2.54	1.33
γ-Cadinene	0.09	0.16	0.31	
Calamenene	0.31	0.28	0.88	0.54
Caryophyllene oxide	0.11		1.56	0.45
Cadinadiene-1,4	0.08		0.59	
Total	96.50	97.24	93.65	97.76

	Fresh leaves		Dri	Dried leaves	
	MGH	HD	MGH	HI	
α-Pinene	0.42	0.77	0.31	0.5	
Sabinene	0.49	0.63	0.36	0.4	
β-Pinene	0.83	1.12	0.67	0.9	
3-Octanol	2.20		0.32	0.1	
α-Terpinen			0.44	0.0	
β-Cymene			0.11	0.0	
Limonen		2.41	1.07	1.0	
1.8-Cineole	6.44	6.78	8.33	6.5	
(E)-β-Ocimene	0.33	-	0.51	0.2	
γ-Terpinen	0.31		0.80	0.1	
c-Sabinene hydrate	1.70	1.37	1.68	2.8	
Terpinolen			0.22	0.0	
t-Sabinene hydrate			0.19	0.0	
Linalyl butyrate	0.58		0.74	0.4	
Isomenthone	27.68	31.92		28.8	
Menthone			14.12	10.7	
Menthofuran	16.35	16.39			
Menthol	24.82	22.00	45.98	29.4	
(-)-4-Terpineol	0.66	1.04			
<i>p</i> -Menth-1-en-8-ol	0.37	0.34	0.84	0.4	
Pulegone	5.80	6.21	4.78	2.7	
(+)-3-Menthene			0.08	0.0	
Dihydroedulan I			0.16	0.0	
Mentyl acetate	0.42		2.22	1.4	
α-Cubebene			0.08	0.0	
β-Bourbonene	0.24	0.21	0.39	0.3	
β-Elemene			0.14	0.1	
β-Caryophyllene	2.92	2.52	3.61	2.7	
β-Farnesene	0.42	0.35	0.79	0.4	
α-Caryophyllene				0.1	
Germacrene D	4.69	4.03	6.27	4.8	
Bicyclogermacrene			1.04	0.9	
β-Cadinene			0.17	0.1	
Hexadecane			0.19	0.1	
γ-Gurjunene	0.49	0.35	0.75	0.9	
Total	98.15	98.43	97.36	98.1	

Structure	Carvone	Limonene	
	CH ₃ H ₃ C CH ₂	CH ₃ H ₃ C CH ₂	
% MGH	65.50	5.87	
% HD	60.27	6.75	
Boiling point (°C)	231	175	
Solubility in water (g/L)	1.6	0.00042	
Dipolar moment (D)	24	14	
Dielectric constant	2.44	0.75	

Table 5: Carvone vs. limonene for M. spicata L.var. rubra: their abundance in EO is related to their physical and chemical characteristics.