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Analytical Methods

Comparison of microwave-assisted hydrodistillation with the traditional hydrodistillation method in the extraction of essential oils from *Thymus vulgaris* L.

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Abstract

Microwave-assisted hydrodistillation (MAHD) is an advanced hydrodistillation (HD) technique utilizing a microwave oven in the extraction process. MAHD of essential oils from the aerial parts (tops) of *Thymus vulgaris* L. (common thyme) was studied and the results were compared with those of the conventional HD in terms of extraction time, extraction yield/efficiency, chemical composition, quality of the essential oils and cost of the operation. MAHD was superior in terms of saving energy and extraction time (75 min, compared to 4 h in HD). Scanning electron microscopy (SEM) of thyme leaves undergone HD and MAHD provided evidences as to a sudden rupture of essential oil glands with MAHD. Gas chromatography–mass spectrometry analysis of the extracted essential oils indicated that the use of microwave irradiation did not adversely influence the composition of the essential oils. MAHD was found to be a green technology.

Keywords: Essential oil composition; Microwave assisted hydrodistillation; Scanning electron microscopy; *Thymus vulgaris* L.

1. Introduction

Thymus vulgaris L. (common thyme), a member of the Labiatae family, is an aromatic/medicinal plant of increasing economic importance for North America, Europe, North Africa and Asia (Letchamo & Gosselin, 1996). Thyme is one of many aromatic plants that has been utilized in variety of food products to provide a flavor specific to this herb (Javanmardi, Khalighi, Kashi, Bais, & Vivanco, 2002; Senatore, 1996; Simon, Morales, Phippen, Vieira, & Hao, 1999; Stahl-Biskup & Sáez, 2002; Stahl-Biskup & Venskutonis, 2004). Studies indicating the antiseptic, carminative, antimicrobial, and antioxidative properties of thyme have also been published (Baranauskiene, Venskutonis, Viskelis, & Dambrauskiene, 2003; Stahl-Biskup & Sáez, 2002; Stahl-Biskup & Venskutonis, 2004). From the medicinal point of view, thyme has been used as a culinary herb and also as a herbal medicine (Stahl-Biskup & Sáez, 2002; Stahl-Biskup & Venskutonis, 2004).

The essential oils of thyme are responsible for the typical spicy aroma of the plant leaves. These oils are stored in glandular peltate trichomes situated on both sides of the leaves (Stahl-Biskup & Sáez, 2002; Stahl-Biskup & Venskutonis, 2004). Results published on the chemical composition of thyme oil revealed that most of the oil was produced from flowering plants (Stahl-Biskup & Sáez, 2002). In the plant's life cycle, the oil production is usually at its highest level during this period. For *T. vulgaris* and *T. pulegioides*, such finding was reported in the early 1960s (Stahl-Biskup & Sáez, 2002).

The main methods to obtain essential oils from the plant materials are hydrodistillation (HD), steam distillation, steam and water distillation, maceration, empyreumatic (or destructive) distillation, and expression (Stahl-Biskup & Sáez, 2002). Among these methods, HD has been the most common approach to extract the essential oils from the

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medicinal herbs/plants (Stahl-Biskup & Sáez, 2002). However, in order to reduce the extraction time and possibly improve the extraction yield, to enhance the quality of the extracts and also to reduce the operation costs, new approaches such as microwave-assisted extraction (MAE), pressurized solvent extraction, supercritical fluid extraction, and ultrasound-assisted extraction have also been sought (Kaufmann & Christen, 2002; Wang & Weller, 2006). As an example, supercritical fluid extraction and supercritical fluid fractionation of thyme oleoresins have been reported in literature (Oszagyan et al., 1996). Some recently-published studies have successfully utilized a microwave oven for the extraction of active components from medicinal plants/herbs (Lucchesi, Chemat, & Smadja, 2004; Stashenko, Jaramillo, & Martinez, 2004a,b; Wang et al., 2006). Lucchesi et al. (2004) reported a solvent-less microwave method for the extraction of essential oils from three aromatic herbs (basil, garden mint, and thyme). The amount of essential oils obtained in 30 min with this method was comparable, both from qualitative and quantitative points of view, to those obtained after 4.5 h HD. In an attempt to take advantage of microwave heating with the conventional HD, microwave-assisted hydrodistillation (MAHD) was then developed and used for the extraction of essential oils from *Xylopia aromatica* (Lamarck) and *Lippia alba* (Mill.) (Stashenko et al., 2004a,b). MAHD was also reported for the extraction of essential oils from *Cuminum cyminum* L. and *Zanthoxylum bungeanum* Maxim (Wang et al., 2006). However, to the best of authors' knowledge no work has been published on the MAHD of thyme species. Therefore, the objectives of this study were to investigate the potential of MAHD for the extraction of essential oils from dried thyme (*T. vulgaris* L.) aerial parts (tops) commonly used in the food, pharmaceutical and cosmetic industries. An attempt has also been made to compare extraction time, extraction yield/efficiency and aromatic composition of the extracts with those of HD.

2. Materials and methods

2.1. Plant materials

Fresh aerial parts of thyme were harvested from open experimental areas of Industrial Research Center for Pesticides and Fertilizers, Karaj, Northern Iran, during the blooming season (late June, 2006). The herbs were then dried under ambient conditions (30–40 °C) for three days on a large screened tray. The identity of the genus thyme was certified by top experts from Pharmacy Department of the University of Tehran, Tehran, Iran. Certified species was then kept in a dark and cold room until used shortly after that for the experiments. Moisture contents of the plants were measured in triplicate according to AACC (1983) method 44-19, using a laboratory oven at 105 °C until constant weight was achieved (7.3%, w/w). All values are reported on a moisture-free basis.

2.2. Microwave-assisted hydrodistillation

A domestic microwave oven (NN-S674MF, Panasonic, Japan, 32 l, 1100 W; variable in 110 W increments, 2.45 GHz) was modified for MAHD operation. The dimensions of the PTFE-coated cavity of the microwave oven were 22.5 cm × 37.5 cm × 38.6 cm. Sixty grams of thyme samples were placed in a 2 l flask containing deionized water (1200 ml). The flask was setup within the microwave oven cavity and a condenser was used on the top (outside the oven) to collect the extracted essential oils (Fig. 1). The microwave oven was operated at 990 W power level for a period of 2 h. This period was sufficient to extract all the essential oils from the sample. During the first 30 min, the collected essential oils were decanted from the condensate in 10 min intervals. Decantation of the essential oils was then continued with 15 min intervals. To remove water, the extracted essential oils were then dried over anhydrous sodium sulfate, weighed and stored in amber vials at 4 °C until they were used for analysis. All the results are reported in grams of essential oils per 100 g of dried thyme aerial parts.

2.3. Hydrodistillation using the conventional clevenger apparatus

HD was carried out in a similar manner as the one explained for MAHD. However, the microwave oven was replaced by an electromantle (EM2000/C, Electrothermal Engineering Ltd., UK, 500 W) and the operation was performed for 4 h (instead of the 2 h period applied for MAHD). Also, the sample collection intervals were increased to 60 min.

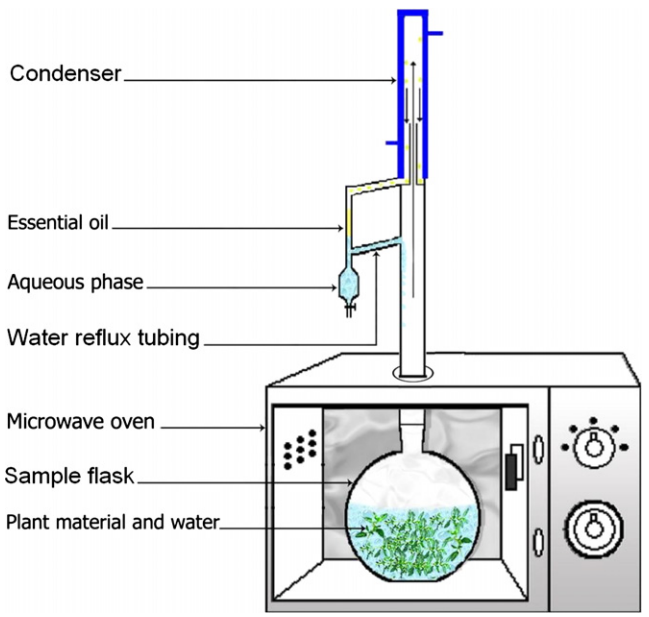

Fig. 1. Schematic representation of the microwave-assisted hydrodistillation apparatus used in this study.

2.4. Physical constants

Specific gravity, refractive index, and color of the essential oils extracted from the thyme samples (by both methods) were measured according to the method suggested by Food Chemical Codex (FCC, 1996). Specific gravity was measured at 25 °C. Refractive index was measured at 20 °C.

2.5. Scanning electron microscopy (SEM)

SEM data of dried thyme leaves were obtained for the untreated samples as well as for those samples undergone MAHD (for 30 min) and HD (for 60 min). The leaves were fixed on the specimen holder with aluminum tape and then sputtered with gold in a sputter coater (BAL-TEC SCD 005, Balzers, Switzerland). All the specimens were examined with a Philips XL 30 scanning electron microscope (Eindhoven, The Netherlands) under high vacuum condition and at an accelerating voltage of 20.0 kV and at a working distance of 8–9 mm.

2.6. Gas chromatography–mass spectrometry (GC–MS)

A GC–MS instrument (5973N, Agilent Technologies, Wilmington, DE, USA) equipped with a mass selective detector operating in the electron impact mode (70 eV) was used to study the compositions of the extracted essential oils. The GC part (6890N, Agilent Technologies, Palo Alto, CA, USA) was equipped with an HP-5MS (Agilent Technologies) capillary column (30 m long, 0.25 mm id and 0.25 μm film thickness). Temperature-programming of the oven included an initial hold at 50 °C for 5 min and a rise to 240 °C at 3 °C min^{-1} followed by additional rise to 300 °C at 5 °C min^{-1}. A final hold for 3 min was allowed for a complete column clean-up.

The injector was set at 290 °C. The samples were diluted with *n*-hexane (1/10, v/v) and a volume of 1.0 μl was injected to the GC with the injector in the split mode (split ratio: 1/10). Carrier gas, He, was adjusted to a linear velocity of 0.8 ml min^{-1}. The compounds of the extracted essential oils were identified by comparing their mass spectral fragmentation patterns with those of similar compounds from a database (Wiley/NBS library) as well as by comparing their Kováts gas chromatographic retention indices (Kováts, 1965) with those of the literature. For each compound on the chromatogram, the percentage of peak area relative to the total peak areas from all compounds was determined and reported as relative amount of that compound.

2.7. Graphite furnace atomic absorption spectroscopy

A GBC atomic absorption spectrophotometer model Avanta PM Version 1.33 (GBC Scientific Equipment Pvt. Ltd., Dandenong, Victoria, Australia) equipped with a Unicam GF3000 electrically heated graphite furnace was used to quantify the lead contents of the extracted essential oils. A Cathodeon hollow cathode lamp of lead operated at 5 mA was used as the radiation source (wavelength: 283.3 nm). The lead content was measured under optimized operating conditions using an air–acetylene flame.

2.8. Statistical analysis

All extractions with HD and MAHD were performed in duplicate and a general linear model (GLM) procedure from SAS (Statistical Analysis Software, version 9.1, SAS Institute Inc., Cary, NC, USA) was used to compare among the means.

3. Results and discussion

3.1. Comparison of extraction kinetics and extraction yield

Kinetics of essential oil extraction from thyme using MAHD has been compared with that of HD on Fig. 2. Extraction with MAHD started at much earlier time than that with HD (7 min vs. 30 min, respectively). This is due to the more efficient heat flow involved with microwaves. Unlike the classical conductive heating methods, microwaves can heat the entire sample almost simultaneously and at a higher rate (Kaufmann & Christen, 2002). Full recovery of essential oils was achieved within the first 2 h of operation with MAHD. In the case of HD, a time period of at least 4 h was necessary for such purpose.

For both HD and MAHD, the extraction starts at the boiling point of water (100 °C, if the operation is performed at atmospheric pressure). With MAHD, the boiling point was reached in 7 min, while in the case of HD this was at 30 min. By the time the extraction of essential oils started with HD, more than 75% of total essential oils (1.89%, w/w) had extracted with MAHD. After 75 min of extraction, MAHD resulted in similar oil recovery to that obtained by 4 h of HD (2.44% vs. 2.39%, respectively).

HD is an approved method that is used as reference for the quantification of essential oils (Stahl-Biskup & Sáez,

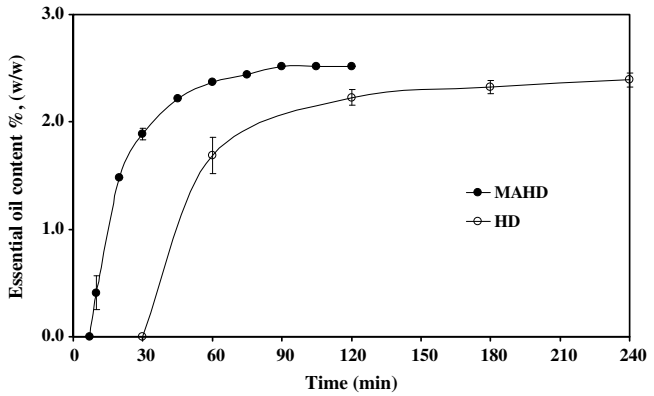

Fig. 2. Extraction yield as a function of time for the hydrodistillation (HD) and microwave-assisted hydrodistillation (MAHD) of essential oils from thyme aerial parts.

2002). The ultimate yield of essential oils extracted by MAHD after 2 h of operation was greater than that obtained by HD after 4 h of operation ($2.52 \pm 0.00\%$ vs. $2.39 \pm 0.06\%$, w/w). These results mean a substantial saving in time.

3.2. Structural changes after extraction

The images from the surfaces of thyme leaves obtained by SEM before and after the extraction are shown on Fig 3. Fig. 3a is a micrograph of the untreated gland (i.e., before the extraction). Images from the thyme leaves undergone a 60 min HD (Fig. 3b) and a 30 min MAHD (Fig. 3c) are also shown for comparison. Both extraction methods resulted in apparent physical changes in the thyme glands. While MAHD destroyed the glands in 30 min, the extraction with HD did not start by that time. This indicates that microwaves (i.e., the irradiation) cause the glandular walls to crumble or rupture more rapidly and more efficiently. The gland undergone HD (Fig. 3b) is wrinkled while that undergone MAHD (Fig. 3c) is not. Such differences can be attributed to a difference in the rate of heat transfer between the two extraction methods. MAHD utilizes three ways of heat transfer within the sample: irradiation, conduction and convection. As a result, with MAHD, heat is produced more quickly from within the glands as well as from the outside (Ferhat, Meklati, Smadja, & Chemat, 2006). With HD, heat transfer can occur through conduction and convection only. For the solvent-free microwave extraction of fresh orange peels, when the glands were subjected to a severe thermal stress and a localized high pressure, as is the case with microwave heating, the pressure build-up within the glands exceeded the capacity of the glands for expansion and as a result a faster rupture of the cell walls was observed, which was not the case with conventional HD (Ferhat et al., 2006).

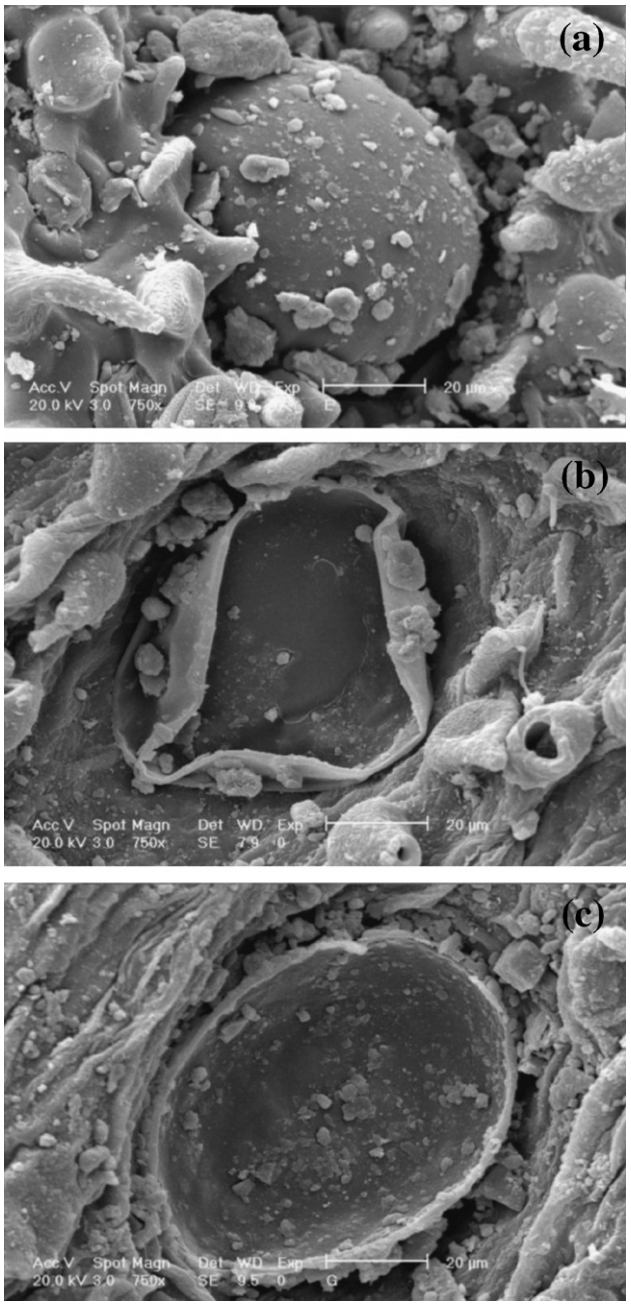

Fig. 3. Scanning electron micrographs of thyme leaves: (a) untreated, (b) after hydrodistillation for 60 min and (c) after microwave-assisted hydrodistillation for 30 min.

3.3. Evaluation of physical properties

Physical properties (appearance, refractive index and specific gravity) of thyme essential oils extracted by both HD and MAHD are shown in Table 1. For comparison purposes, standard properties obtained from FCC (1996) are also shown in the same Table. The refractive indices and specific gravities of essential oils obtained from thyme aerial parts for both MAHD and HD fall within the ranges specified by FCC. Similarly, the colors of both essential oils are in the range indicated by FCC. The only difference was that the color of the essential oils extracted by MAHD was lighter than that obtained by HD. Therefore, considering physical properties of the extracted essential oils, MAHD as a new extraction technique does not introduce any problems to the essential oils extracted from the aerial parts of thyme.

3.4. Lead content

The amounts of lead extracted along with the essential oils were 19.80 ± 0.67 μg kg^{-1} for HD and 66.40 ± 6.40 μg kg^{-1}

Table 1
Physical properties of essential oils from thyme aerial parts obtained by hydrodistillation (HD) and microwave-assisted hydrodistillation (MAHD)

Physical properties	FCC[a]	HD	MAHD
Specific gravity (25 °C)	0.915–0.935	0.918	0.924
Refractive index (20 °C)	1.495–1.505	1.503	1.503
Appearance	A colorless, yellow, or red liquid	Yellow	Pale yellow

[a] Standard physical properties for thyme essential oils from FCC (1996).

for MAHD. Although this quantity was greater for the essential oils extracted by MAHD, its amount was still below the maximum permitted lead content of thyme essential oils according to FCC (0.02%). Due to the higher extraction rate by MAHD and also because of severe thermal stresses and localized high pressures involved with such technique (Lucchesi et al., 2004; Ferhat et al., 2006), MAHD resulted in greater amounts of lead in the extracted essential oils from thyme aerial parts compared to that in HD.

3.5. Gas chromatography–mass spectrometry

The identities of the extracted essential oils from both methods are shown in Table 2. The 29 components shown in Table 2 consist of about 95% of total GC peak area. The compositions of the essential oils obtained by both HD and MAHD were similar and as a result all components extracted by HD were also found in MAHD. Except for γ-terpinene (compound 12) and α-terpineol (compound 19), there were no significant differences between the quantities of the extracted components by HD and those extracted by MAHD. Both essential oils were found to be rich in the active monoterpene phenols (thymol and carvacrol) and their corresponding monoterpene hydrocarbon precursors (*p*-cymene and γ-terpinene). Thymol (compound 22) was the most abundant component in thyme essential oil (37.20% for HD and 40.20% for MAHD) followed by *p*-cymene (compound 10), γ-terpinene (compound 12) and carvacrol (compound 23). This is in agreement with the values previously reported for the same species, but using conventional extraction methods (Stahl-Biskup & Sáez, 2002; Stahl-Biskup & Venskutonis, 2004). Therefore, microwave does not involve in any deterioration of the extracted components and it can be introduced as a safe method for the extraction of essential oils.

Table 2
Chemical compositions of essential oils obtained by hydrodistillation (HD) and microwave-assisted hydrodistillation (MAHD) of thyme aerial parts

No.	RT[b] (min)	Compound	I_K[c]	Relative peak area[a] (%)	
				HD	MAHD
1	16.05	α-Thujene	930	0.63 ± 0.13	0.53 ± 0.02
2	16.53	α-Pinene	938	1.02 ± 0.17	0.86 ± 0.01
3	17.31	Camphene	952	0.58 ± 0.11	0.53 ± 0.01
4	19.04	1-Octen-3-ol	983	2.69 ± 0.16	2.64 ± 0.31
5	19.60	β-Myrcene	993	1.41 ± 0.21	1.30 ± 0.17
6	19.91	3-Octanol	999	0.20 ± 0.02	0.19 ± 0.03
7	20.39	α-Phellandrene	1008	0.20 ± 0.02	0.18 ± 0.01
8	20.71	Δ-3-Carene	1014	0.11 ± 0.01	0.09 ± 0.01
9	21.12	α-Terpinene	1021	1.92 ± 0.15	1.73 ± 0.14
10	21.73	*p*-Cymene	1033	16.85 ± 0.08	17.57 ± 0.78
11	21.99	1,8-Cineole	1037	1.36 ± 0.08	1.31 ± 0.12
12	23.55	γ-Terpinene	1066	9.06 ± 0.12	8.54 ± 0.02
13	23.91	*Trans*-Sabinene hydrate	1073	1.09 ± 0.02	0.94 ± 0.05
14	24.95	Terpinolene	1093	0.26 ± 0.01	0.27 ± 0.05
15	25.59	Linalool	1105	2.50 ± 0.14	2.43 ± 0.27
16	29.20	Borneol	1176	1.19 ± 0.03	1.11 ± 0.21
17	29.69	Endo-Borneol	1185	1.86 ± 0.25	1.41 ± 0.21
18	29.80	Terpinen-4-ol	1187	0.71 ± 0.05	0.63 ± 0.16
19	31.20	α-Terpineol	1216	0.26 ± 0.00	0.17 ± 0.00
20	32.34	Methyl thymylether	1240	0.16 ± 0.02	0.14 ± 0.07
21	34.32	Geraniol	1281	0.34 ± 0.05	0.39 ± 0.07
22	35.90	Thymol	1315	37.20 ± 2.86	40.20 ± 3.03
23	36.50	Carvacrol	1328	6.81 ± 0.05	6.84 ± 0.68
24	38.22	Thymyl acetate	1366	0.14 ± 0.02	0.16 ± 0.03
25	41.36	β-Caryophyllene	1438	3.05 ± 0.20	2.86 ± 0.27
26	42.72	α-Humulen	1470	0.603 ± 0.00	0.64 ± 0.22
27	42.98	Geranyl acetate	1477	0.39 ± 0.02	0.42 ± 0.09
28	45.44	Δ-Cadinene	1536	0.40 ± 0.03	0.40 ± 0.05
29	48.17	Caryophyllene oxide	1604	1.34 ± 0.12	1.42 ± 0.21
Total peak area (%)				94.31	95.91
Total extraction time (min)				120	240
Yield (%)				2.39 ± 0.06%	2.52 ± 0.00%

[a] Mean ± SD ($n = 2$).
[b] Retention time.
[c] Kováts Retention Index (I_K) relative to C_9–C_{18} *n*-alkanes on the HP-5MS column.

3.6. Cost, cleanliness and scale-up

As shown in Fig. 2, HD required an initial time period of 30 min for heating 1.2 l of water containing 60 g sample until the extraction started. Additional time required for the full extraction of essential oils was 210 min. However, MAHD required only 7 min for the initial stage and additional 68 min for the final stage. Therefore, MAHD can result in significant saving in the extraction time. Furthermore, the energy requirements to perform the extraction, based on the maximum power consumption of the electromantle for HD and microwave oven for MAHD considering the total period of a full recovery, were 2.00 kWh for HD and 1.24 kWh for MAHD. This indicates a substantial saving in the extraction cost when using MAHD instead of HD.

Assuming the typical generators for the production of electricity, which used to be the major source of electricity for a long time, the equivalent quantities of carbon dioxide released to the atmosphere for the production of 1 g essential oils were estimated according to Ferhat et al. (2006) at 1600 g CO_2 level for HD and 990 g for MAHD. Therefore, MAHD can be suggested as an "environmentally friendly" extraction method, which avoids the use of organic solvents typical to Soxhlet and ultrasound-assisted extractions as well as accelerated solvent extraction. MAHD can also be offered for the production of larger quantities of essential oils by applying the existing large-scale microwave extractors instead of the conventional hydrodistillation extractors.

4. Conclusions

MAHD offered substantial advantages over conventional HD. A similar extraction yield was achieved at significantly shorter extraction time when using MAHD instead of HD. Therefore, considering the operation cost,

MAHD could be carried out using half of the expenses required by HD.

SEM images of thyme aerial parts undergone MAHD and HD indicated that microwaves cause a quick rupture of the glandular walls resulting in a higher extraction efficiency at a shorter time. GC–MS results indicated that there were no significant differences between the essential oils obtained by MAHD and those obtained by HD proposing MAHD as an excellent alternative for HD with no adverse effects on the composition of the extracted essential oils.

Significantly lower energy consumption with MAHD (~38%) renders this technology being more environmentally friendly than HD. Compared to many solvent extraction techniques such as Soxhlet and accelerated solvent extraction, MAHD is modern, green and fast.

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