Food Chemistry 114 (2009) 355–362

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Comparison of two isolation methods for essential oil from rosemary leaves: Hydrodistillation and microwave hydrodiffusion and gravity

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ARTICLE INFO

Article history:
Received 7 May 2008
Received in revised form 25 September 2008
Accepted 27 September 2008

Keywords:
Microwave
Hydrodiffusion
Antioxidant
Essential oil
Rosemary

ABSTRACT

Traditional hydrodistillation (HD) and innovative Microwave Hydrodiffusion and Gravity (MHG) methods have been compared and evaluated for their effectiveness in the isolation of essential oil from fresh *Rosmarinus officinalis* leaves. The microwave method offers important advantages over traditional alternatives, namely: shorter isolation times (15 min against 3 h for hydrodistillation), environmental impact (energy cost is fairly higher to perform HD than that required for rapid MHG isolation), cleaner features (as no residue generation and no water or solvent used), increases antimicrobial activities, increases antioxidant activity and provides a more valuable essential oil (with high amount of oxygenated compounds). It offers also the possibility for a better reproduction of natural aroma of the essential oil from rosemary leaves than the HD essential oil. Moreover, microwave procedure yielded essential oils that could be analysed or used directly without any clean-up, solvent exchange or centrifugation steps. Scanning electron microscopy shows important structural changes for MHG extraction in contrast to those obtained by HD. Electron micrographs show clearly that the cells are broken and damaged during microwave treatment. Finally, the mechanism of Microwave Hydrodiffusion and Gravity is proposed and discussed.

1. Introduction

Rosemary (*Rosmarinus officinalis* L.) is a perennial herb with fragrant evergreen needle-like leaves. It is native to the Mediterranean region and it has been cultivated for a long time. It belongs to the Lamaiaceae family, which comprises up to 200 genera and about 3500 species. The leaves are evergreen, with dense short woolly hairs. Rosemary has been a significant herb since antiquity, although rosemary is more familiar to contemporary Westerners as a kitchen herb used to add a spicy or slightly medicinal flavour to some foods, it was traditionally used as an antiseptic, astringent, and food preservative before the invention of refrigeration. Rosemary's antioxidant properties are still used to extend the shelf life of prepared foods (Cuvelier, Richard, & Berest, 1996). Rosemary is also known medicinally for its powerful antioxidant activity (Ibanez et al., 2003), its antibacterial and antimutagenic properties, and as a chemopreventive agent (Oluwatuyi, Kaatz, & Gibbons, 2004). Besides the therapeutical application, the essential oil is widely applied in the cosmetic industry producing various Cologne waters, bathing essences, hair lotions and shampoos and as a component of disinfectants and insecticides (Boelens, 1985).

The essential oil secreted by glandular trichomes is mainly located in leaves. Essential oil can be isolated using a number of isolation methods, e.g. hydrodistillation, steam distillation and organic solvent extraction. Nevertheless, monoterpenes are well known to be vulnerable to chemical changes under steam distillation conditions, and even conventional solvent extraction is likely to involve losses of more volatile compounds during removal of the solvent (Presti et al., 2005). Many of these methods are moreover time-consuming and energy intensive. There are many publications dealing with the extraction of *R. officinalis* using alternative techniques. Recently, the supercritical fluid extraction of rosemary with CO_2 has been the object of a lot of research (Carvalho, Moura, Rosa, & Meireles, 2005) and has become a valid alternative to the more conventional extraction procedures, mainly because the dissolving power of the extracting medium can be adjusted by regulating the pressure and temperature conditions. However, the technological conditions required for the use of supercritical fluid extraction are onerous and the high cost of producing specific products has limited its use. Moreover, in certain cases, the extractive power of supercritical CO_2, towards specific analytes, is insufficient under conventional conditions (Lucien & Foster, 2000) and

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doi:10.1016/j.foodchem.2008.09.106

inversely excessive with the extraction of undesirable compounds as vegetable waxes or resins (Guinamant, 1992). Microwave-assisted solvent extraction (MASE) (Chen & Spiro, 1995) appeared to be particularly attractive for isolation of essential oil from rosemary. The popularity of microwave technique is due to the rapid rates of heat transfer which allows quicker times of extraction. Ultrasounds were also used to increase the solvent extraction efficiency of antioxidants from rosemary (Albu, Joyce, Paniwnyk, Lorimer, & Mason, 2004). The application of ultrasound irradiation facilitated the low-temperature rupturing of plant cell membranes, thereby liberating molecules from cellular structures. However, the principal drawback of these methods is that the solvent used cannot be completely separated from the extract at the end of the process. Tigrine-Kordjani, Meklati, and Chemat (2006) have been recently developed a microwave-assisted distillation (MAD) with free solvent for laboratory scale applications in the extraction of essential oils from different kinds of aromatic plant. Recently, Bendahou et al. (2008) have been reported the extraction of *Origanum glandulosum* essential oil with MAD. Nowadays, more and more advanced techniques utilising microwave radiations in the extraction process have been documented in the literature (Golmakani & Rezaei, 2008; Wang et al., 2008). Rezzoug, Boutekedjiret, and Allaf (2005) have been isolated essential oil from rosemary leaves by an innovative process called "Detente Instantannée controlée" or controlled instantaneous decompression. This process involves subjecting plant material for a short time to a steam pressure followed by an instantaneous decompression to a vacuum.

Recently, a new greener extraction technique, Microwave Hydrodiffusion and Gravity was designed and developed. The essential oil isolation based on this technique, which was successfully tested for isolation of essential oil from mint plants (Abert Vian, Fernandez, Visinoni, & Chemat, 2008) is an interesting alternative not only to standard techniques of essential oil isolation, such as isolation with solvent or steam distillation, but also to more effective processes described above. This process is based of an original combination of microwave heating and earth gravity at atmospheric pressure.

In this paper, the essential oil from rosemary (*R. officinalis*) obtained by Microwave Hydrodiffusion and Gravity has been compared with those obtained by conventional hydrodistillation. We make appropriate comparisons in terms of kinetics of isolation, quality and quantity of essential oil, antimicrobial activities, antioxidant activity and energy consumption. We have also proposed a mechanism for MHG technique. This study was supplemented by scanning electron micrographs to shed light on the isolation mechanism.

2. Experimental

2.1. Plants material

Leaves of the cultivated plants of rosemary (*R. officinalis* L.) were collected in 2007 from the Institut National Agronomique (INA-El Harrach – Alger). The initial moisture of leaves was 60.2%.

2.2. MHG apparatus and procedure

Microwave Hydrodiffusion and Gravity has been performed using the "DryDist" microwave oven illustrated in Fig. 1. This is a multimode microwave reactor 2.45 GHz with a maximum delivered power of 1000 W variable in 10 W increments. Temperature was monitored by an external infrared sensor. In a typical MHG procedure performed at atmospheric pressure, 500 g of fresh plant material were heated using a fixed power density of 1 W g^{-1} for 15 min without addition of solvent or water.

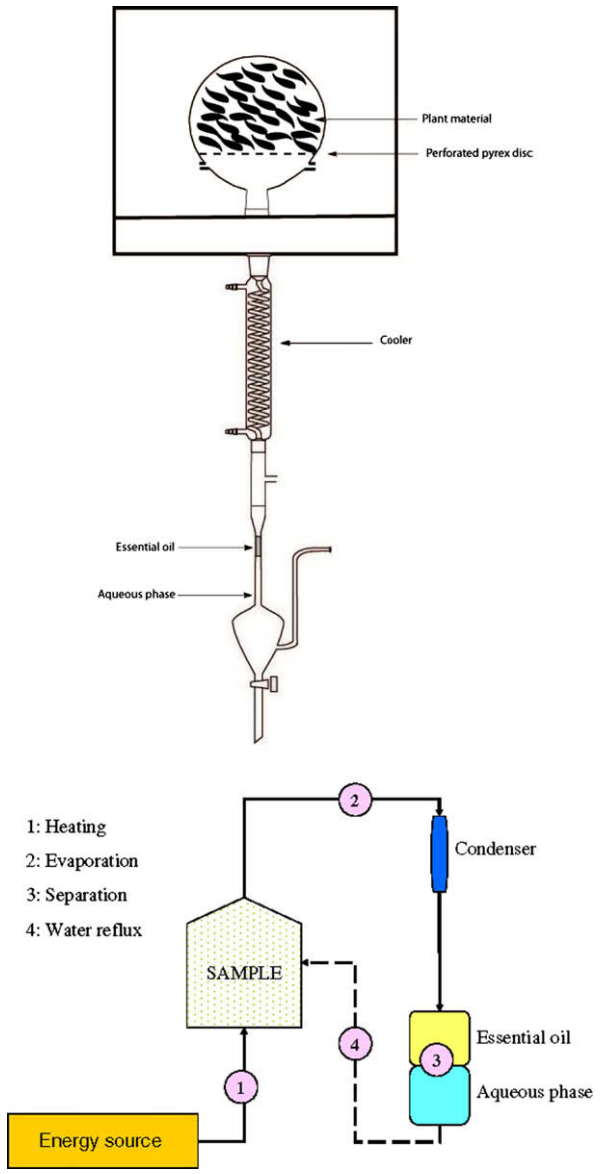

Fig. 1. Isolation methods of essential oil from *Rosmarinus officinalis* (MHG and HD).

The direct interaction of microwaves with biological water (i.e., steam produced from the water present in the fresh plant material) favours the release of essential oils trapped inside the cells of plant tissues. A mixture of hot "crude juice" and steam (*in situ* water) move thus naturally by earth gravity downwards on a spiral condenser outside the microwave cavity where it condensed. The oily condensate is collected continuously in a receiving flask (similar to separator funnel) where essential oil forms a film on the surface of the water and the film is skimmed off the top. At the end the essential oil is collected, dried with anhydrous sodium sulphate and stored at 4 °C until used.

2.3. Hydrodistillation apparatus and procedure

Five hundred grams of each aromatic herb were submitted to hydrodistillation with a clevenger-type apparatus (Conseil de l'Europe and Pharmacopée Européenne 1, 1996) according to the European Pharmacopoeia and extracted with 3 l of water for 90 min (until no more essential oil was obtained). The essential oil was collected, dried under anhydrous sulphate and stored at 4 °C until used.

2.4. GC and GC–MS identification

GC analysis was carried out using a Hewlett–Packard 6890N gas chromatograph equipped with a flame ionisation detector (FID), under the following operation conditions: vector gas, helium; injector and detector temperatures, 250 °C; injected volume, 1 μl; split-less mode; HP5MS™ (30 m × 0.25 mm I.D., film thickness 0.25 μm; constant flow 0.3 ml/min) and Carbowax™ poly(ethylene glycol) (60 m × 0.20 mm I.D., film thickness 0.25 μm); the oven temperature programme was 40 °C for 8 min increased at 2 °C/min to 250 °C and held at 250 °C for 30 min.

Retention indices were determined with C_5–C_{26} alkane standards as reference. Relative amount of individual components are based on peak areas obtained without FID response factor correction. Three replicates were performed for each sample. The average of these three values and the standard deviation were determined for each component identified.

GC–MS analysis was carried out using an Agilent 6890N coupled to an Agilent 5973 MS. Samples were analysed on a fused-silica capillary column HP5MS™ (50 m × 0.20 mm I.D., film thickness 0.50 μm) and Carbowax™ poly(ethylene glycol) (60 m × 0.20 mm I.D., film thickness 0.25 μm). Carrier gas, helium; injector and detector temperatures, 250 °C; injected volume, 1 μl; split-less mode; the oven temperature programme was 40 °C for 8 min increased at 2 °C/min to 250 °C and held at 250 °C for 30 min; ionisation energy, 70 eV; electron ionisation mass spectra were acquired over the mass range 35–400 μm.

Identification of the components was based on computer matching against commercial libraries (Wiley, MassFinder 2.1 Library, NIST98), laboratory mass spectra libraries built up from pure substances, and MS literature data (Joulain & König, 1998; Joulain, König, & Hochmuth, 2001; McLafferty & Stauffer, 1989; Adams, 1995; B. A. C. I. S., 1999) combined with comparison of GC retention indices (RI) on apolar and polar column. RIs were calculated with the help of a series of linear alkanes C_6–C_{26} on apolar and polar columns (HP5MS™ and Carbowax™). Compounds available in the laboratory were confirmed by external standard compound co-injection.

2.5. Scanning electron microscopy (SEM)

The specimens were freeze-dried, fixed on the specimen holder with aluminium tape and then sputtered with gold. All the specimens were examined by a Philips XL30, under vacuum condition and accelerating voltage of 10 kV, with a spot size 5 and a working distance of 15 mm.

2.6. Physical constants

Rosemary essential oils have been analysed according to the standard method AFNOR. The usual physical constants defining the essential oil have been determined at 20 °C: specific gravity, refractive index and optical rotation.

2.7. Sensory evaluation

The sensory evaluation of essential oils was conducted by twelve trained panelists who were graduate students and staff members in the laboratory of the University of Avignon. Randomly coded samples were individually served to panelists.

2.8. Screening for antimicrobial activity

The paper disc diffusion method was employed to determine the antimicrobial activity of the essential oils. For these assays the cultures of the following micro-organisms were used: one gram-positive (*Staphylococcus aureus*) and one gram-negative (*Escherichia coli*) bacteria and one yeast (*Saccharomyces cerevisiae*). All micro-organisms were supplied from the Algerian pharmaceutical industry SAIDAL. Cultures of the micro-organisms were maintained on nutrient agar (NA) medium. Briefly, a suspension of the tested micro-organism (10^7–10^8 CFU/ml) was spread on the solid media plates. Filter paper discs of 6 mm diameter (Whatman no. 1) were individually impregnated with 50 ml of essential oil, then laid on to the surface of the inoculated plates. At the end of incubation time (24 h at 37 °C for bacteria, 48 h at 25 °C for yeasts), positive antibacterial and antifungal activities were established by the presence of measurable zones of inhibition. The antimicrobial activity was recorded as the width (in millimetres, diameter of the disc included) of the zone of inhibition after incubation. Each test was performed in three replicates and repeated twice.

2.9. Screening for antioxidant activity

Antioxidant activity of essential oils was determined using the photochemiluminescence (PCL) in which the photochemical generation of free radicals is combined with the sensitive detection by using chemiluminescence. This reaction is induced by optical excitation of a photosensitizer S which results in the generation of the superoxide radical $O_2^{\cdot-}$ (Popov & Lewin, 1999)

$$S + h\nu + O_2 \rightarrow [S * O_2] \rightarrow S^{\cdot+} + O_2^{\cdot-}$$

The free radicals are visualised with the chemiluminescent detection reagent luminol. It works as photosensitizer as well as oxygen radical detection reagent. The essential oils were measured in the Photochem® with the ACL kit (AnalytikJena, Jena, Germany). A 2.2 ml portion of reagent 1 (solvent and dilution reagent), 200 μl of reagent 2 (buffer solution), 25 μl of reagent 3 (photosensitizer), and 100 μl of standard (trolox reagent in reagent 1) or sample (essential oil in methanol) solution were mixed and measured. A light emission curve was recorded over 180 s, using inhibition as the parameter to evaluate antioxidant potential. The antioxidant capacity was then determined by using the integral under the curve and was expressed as mmol/l of trolox used as standard to obtain calibration curve.

3. Results and discussion

3.1. Kinetics microwave extraction

The yields of essential oil extracted from rosemary (*R. officinalis*) with the different isolation methods are respectively 0.35 ± 0.07% and 0.33 ± 0.09% for the HD and MHG. As is shown in Fig. 2a and b, an isolation time of 15 min with MHG provides yields similar to those obtained after 180 min by means of HD, which is one of the reference methods in essential oil isolation.

Fig. 2a and b shows the variation of the extraction yield according to the extraction time. Three phases are observed in the process of the microwave extraction (Fig. 2a). Step 0 represents the heating phase from room temperature to 100 °C. The first step (Step 1) is represented by an increasing line which characterises the first quantities extracted, located at the surface of vegetable particles representing approximately 82% of the yield obtained into 5.5 min. This phase is followed by an second increasing line (Step 2) representing the intern diffusion of the essential oil from the midst of the particles towards the external medium involved by the intern warming of the water located in the plant cells. In this stage (realised into 8 min), the oil amount extracted represents nearly 18% of the global yield.

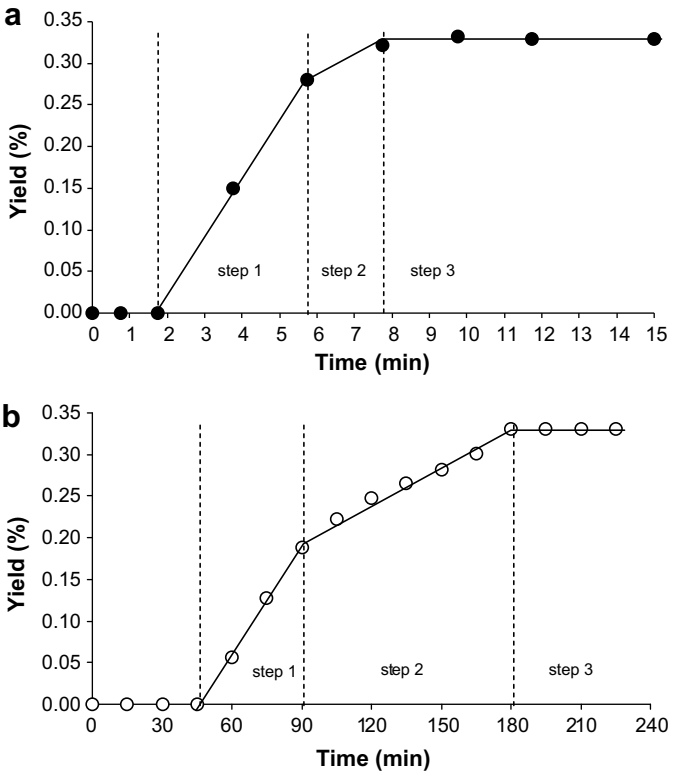

Fig. 2. Yield profiles as a function of time for the MHG (a) and HD (b) isolations of essential oil from rosemary leaves.

The third part (Step 3) corresponds to a horizontal line which marks the end of the extraction process. Benkaci-Ali, Baaliouamer, and Meklati (2006) using microwave extraction of essential oil from *Nigella sativa* L. seeds, found a yield profile similar to that described here. The profile of the conventional extraction technique HD presents three similar aspects but different phases to those obtained with MHG; the first step leading to 60% of the yield obtained into 93 min. The end of the extraction process is reached after 180 min.

3.2. Optimization of microwave power

An appropriate microwave irradiation power is important to ensure the essential oil is extracted quickly; however, the power should not be too high otherwise loss of volatile compounds would result. Different microwave irradiation power, 200, 400, 600, 800, and 1000 W, were examined for MHG extraction of essential oils. The total extraction time (it was until no more essential oil was obtained) in relation with the microwave irradiation power was studied. A microwave irradiation power of 500 W for 500 g of plant material was the optimum microwave power density because this power permits in only 15 min to extract the essential oil completely and avoid loss of volatile compounds.

3.3. Quality and quantity of essential oils

A total of 33 compounds (Table 1) were identified in rosemary essential oils using the two techniques. MHG and HD enabled the detection of most volatile active compounds in rosemary essential oil such as α-pinene, camphor, verbenone and camphene, but their proportions depends on the isolation technique. Lightly higher amounts of oxygenated compounds are present in the essential oils of the aromatic plant isolated by MHG in comparison with HD. The monoterpene hydrocarbons (α-pinene ...) are present in equivalent amounts in the HD and MHG essential oils, but the oil obtained by MHG is a little more concentrated in oxygenated compounds. The essential oil of rosemary leaves isolated either by MHG or HD contains the same dominant components. α-Pinene, a monoterpene hydrocarbon, is the main abundant component in the essential oil extracted from *R. officinalis* with equivalent relative amounts for both extraction methods: 43.6% and 44.05%, respectively for MHG and HD.

Camphor was the main oxygenated component in the essential oil isolated from rosemary leaves but the relative amounts differed for the two isolation methods. It is the most abundant oxygenated component of the MHG oil 8.6%, whereas the HD oil contains 7.82%.

Monoterpene hydrocarbons are less valuable than oxygenated compounds in terms of their contribution to the fragrance of the essential oil. Conversely, the oxygenated compounds are highly odoriferous and, hence, the most valuable. The greater proportion of the detected compounds and the proportion of the oxygenated

Table 1
Chemical composition of *Rosmarinus officinalis* essential oils obtained by MHG and HD.

No.	Compounds[a]	HD (%)	MHG (%)	RI[b]	RI[c]
	Monoterpene hydrocarbons	68.56	68.60		
1	Tricyclene	0.26	0.26	921	1011
2	α-Pinene	44.05	43.60	936	1023
3	Camphene	6.14	6.48	951	1103
4	Verbenene	0.77	1,11	955	1121
5	β-Pinene	2.61	2,20	980	1109
6	Myrcene	1.94	1,82	995	1149
7	α-Phellandrene	0.31	0.34	995	1165
8	γ-3-Carene	0.08	0.1	1014	1290
9	α-Terpinene	0.86	1.00	1020	1083
10	para-Cymene	1.27	1.50	1025	1250
11	Limonene	5.48	5.53	1030	1206
12	γ-Terpinene	3.08	3.01	1052	1251
13	Terpinolene	1.71	1.65	1092	1287
	Oxygenated monoterpenes	24.87	28.10		
15	Linalool	2.00	2.39	1106	1538
16	α-Campholenal	1.24	1.32	1122	1471
17	Camphor	7.82	8.60	1149	1514
18	Pinocarvone	1.33	1.81	1160	1548
19	Borneol	2.57	2.71	1173	1679
20	Terpin-4-ol	2.07	2.11	1184	1590
21	α-Terpineol	0.77	0.87	1198	1677
22	Verbenone	6.37	7.65	1207	1696
23	Geraniol	0.70	0.64	1279	1828
	Sesquiterpene hydrocarbons	1.91	1.41		
24	E-Caryophyllene	0.95	0.76	1425	1470
25	α-Humulene	0.42	0.	1450	1657
26	γ-Curcumene	0.04	0.04	1469	1738
27	β-Bisabolene	0.43	0.22	1508	1714
28	β-Sesquiphellandrene	0.07	0.04	1519	1776
	Oxygenated sesquiterpenes	0.26	0.25		
29	Caryophyllene oxide	0.10	0.11	1570	1977
30	α-Bisabolol	0.16	0.14	1684	2022
	Other oxygenated compounds	1.03	1.19		
31	Bornyl acetate	0.81	0.95	1263	1579
32	Methyl eugenol	0.12	0.13	1397	2032
33	Z-Methyl jasmonate	0.10	0.11	1635	2349
Extraction time (min)		180	15		
Yield (%)		0.35 ± 0.07	0.33 ± 0.09		
Total oxygenated compounds		26.16	29.54		
Total non-oxygenated compounds		70.47	70.01		

[a] Essential oil compounds sorted by chemical families and percentages calculated by GC–FID on non-polar HP5MS™ capillary column.
[b] Retention indices relative to C_5–C_{28} *n*-alkanes calculated on non-polar HP5MS™ capillary column.
[c] Retention indices relative to C_5–C_{28} *n*-alkanes calculated on polar Carbowax™-PEG capillary column.

Table 2
Properties (physical and sensorial) and activities (anti-microbial and antioxidant) of rosemary essential oils obtained by MHG and HD.

	MHG	HD
Physical properties		
Specific gravity	0.9	0.9
Refractive index	+1.468	+1.470
Optical rotation in degree	+2	+3
Sensory evaluation		
Colour	Pale yellow	Pale yellow
Odour	Fresh, light, freshly camphorated with a slight note of citrus, odour closed to fresh aromatic herb	Freshly camphorated and citrus, boiled odour, different from fresh fruit
Aspect	Liquid movable	Liquid movable
Anti-microbial activity	Inhibition zone diameter (mm ± standard deviation) for tested micro-organism	
Staphylococcus aureus (*Staphlococcaceae*) Gram-positive bacteria (ATCC 6538)	17 ± 0.5	12.5 ± 0.4
Escherichia coli (*Enrerobacteriaceae*) Gram-negative bacteria (ATCC 4157)	19.0 ± 0.5	15.5 ± 0.5
Saccharomyces cerevisia (*Saccharomycetaceae*) Yeast (ATCC 2601)	24.0 ± 0.6	20.0 ± 0.6
Anti-oxidant activity	Photochemiluminescence expressed as mmol equivalents of trolox per litre of sample ± standard deviation	
mmol Trolox/l	4.53 ± 0.02	3.68 ± 0.06

compounds in MHG essential oils are probably due to the diminution of thermal and hydrolytic effects, relatively to HD which uses a large quantity of water. Essential oils contain organic compounds that strongly absorb microwave energy. Compounds with high and low dipolar moments could be extracted in various proportions by microwave extraction. Organic compounds like oxygenated compounds that have a high dipolar moment will interact more vigorously with microwaves and can be extracted more easily in contrast with aromatic compounds which have low dipolar moments (like monoterpene hydrocarbons). Rosemary essential oils have been analysed according to the standard method AFNOR to determine the usual physical constants defining the essential oil extracted either by MHG and HD: specific gravity, refractive index, and optical rotation. There is no significant difference between the physical constants of essential oils obtained by these methods.

The sensory evaluation of essential oils extracted by MHG and HD are shown in Table 2. MHG method offers the possibility for a better reproduction of natural aroma of the aromatic plant essential oil than the hydrodistilled essential oil. MHG could be a good alternative for the isolation of essential oils from rosemary leaves.

3.4. Antimicrobial activity

The antimicrobial activity of the essential oils from rosemary leaves, isolated by two isolation methods, against three species of micro-organisms by the disc diffusion method was recorded in Table 2. The essential oils showed inhibition zones against all micro-organisms tested. The data obtained from disc diffusion method using rosemary essential oil, indicated that *S. cereveciae* was the most sensitive micro-organism tested with the largest inhibition zone (24–20 mm) and *S. aureus* exhibited the smallest inhibition zone (17–12.5 mm). Overall, the essential oils displayed a broad antimicrobial spectrum and exerted a stronger antimicrobial effect against gram-positive bacteria than gram-negative bacteria. The antimicrobial activity of the rosemary essential oils obtained by MHG is slightly higher than that obtained with HD. The antimicrobial activity of MHG essential oil can be linked to its higher content of oxygenated compounds.

3.5. Antioxidant activity

Photochemiluminescence is a modern technique for the estimation of the total antioxidant capacity. It is based on an antioxidant-sensitive inhibition of a photo-induced, chemiluminescence accompanied auto oxidation of luminol. The luminol is a photosensibilitiser, generating superoxide, radicals, and also a chemiluminogenic probe for free radicals. Because superoxide radical is a deleterious by-product of oxygen metabolism, responsible for the most important diseases, the values obtained by the PCL method give an evaluation of the protective capacity of a given ingredient against ROS which are the most dangerous species of free radicals for leaving beings.

Data obtained from PCL testing show very small differences between the antioxidant activities of the oils obtained with each extraction technique. In this assay, rosemary essential oil obtained by MHG showed a slightly better antioxidant capacity value, which is equivalent to 4.53 ± 0.02 mmol of trolox per litre of sample, than the essential oil obtained by HD with 3.68 ± 0.02 mmol of trolox per litre of sample (Table 2). These small differences could be due to the higher proportion of the oxygenated compounds contained in MHG essential oil.

3.6. Structural changes after extraction

The different extraction methods (MHG and HD) produce distinguishable physical changes in the plant material. Fig. 3a–f enable comparison of scanning electronic micrographs of rosemary. The structures of the untreated plant material can be compared with those of the material treated by MHG or HD. The untreated rosemary leaf shows glandular trichomes which contain volatile oils; amongst glandular trichomes, two types are recognised – peltate and capitate trichomes. The peltate trichomes (Fig. 3b) were predominantly on the abaxial surface and located in epidermal depressions and they consisted of one basal epidermal cell. The capitate trichomes (Fig. 3a) were situated on the adaxial and abaxial leaf surface, and they were more numerous than peltate trichomes. They consisted of a basal cell, a short mono or bicellular stalk and a rather large one or two celled secretory head.

After MHG (15 min only), cells and cell walls have been affected and it can be observed damages of the oil-containing glands. Glandular trichomes (Fig. 3c and d) seems to be destroyed. In contrast, plant material subjected to hydrodistillation (3 h) appeared very similar to untreated material. It can be seen a partial destruction of secreting glands and a part of them are still filled (Fig. 3e and f).

The changes observed for MHG extraction were markedly different from those observed by HD, showing clearly that the cells are broken and damaged during microwave treatment. This indicates that the mechanical strain induced by the rapid decompression and the violent vaporisation of water have two main effects – the dehydrating effect of vaporisation and a subsequent

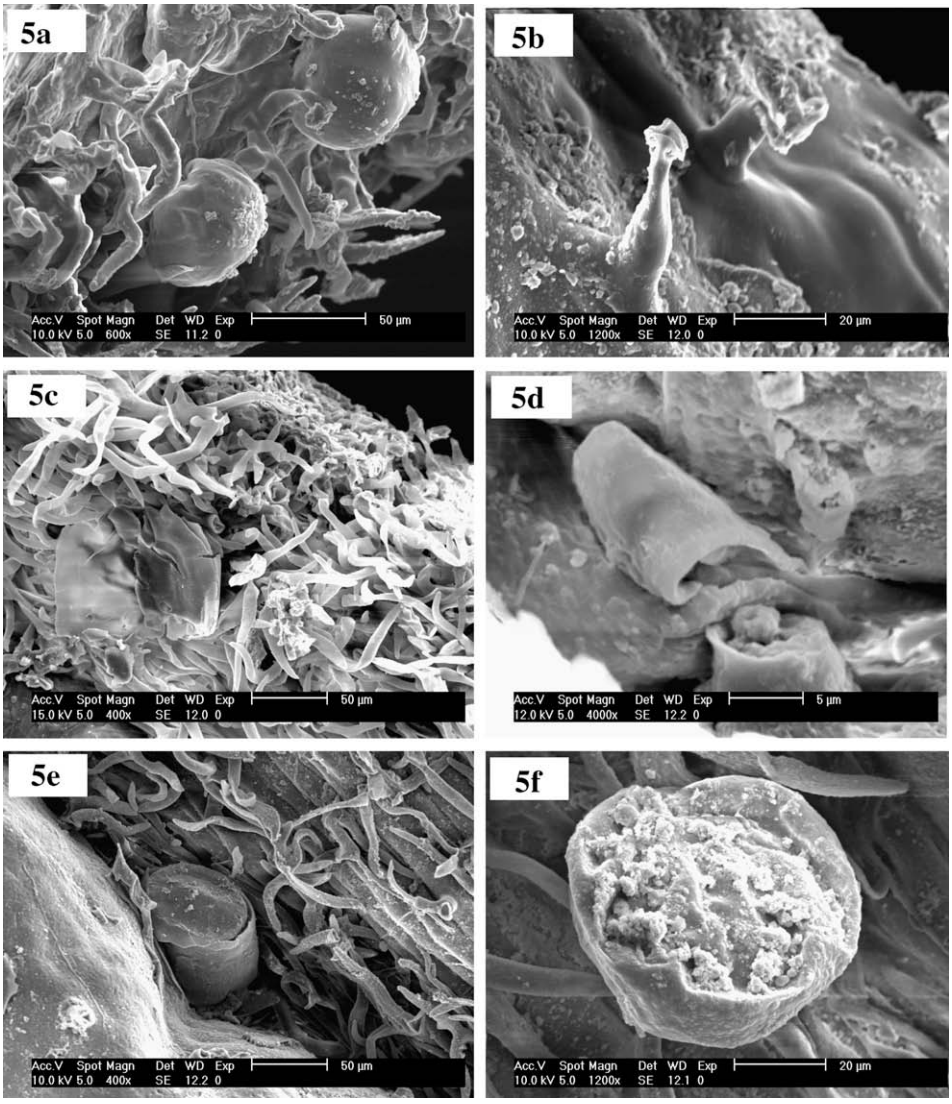

Fig. 3. Electron micrographs of rosemary leaves: (a) capitate trichomes of untreated; (b) peltate trichomes of untreated; (c) capitate trichomes after MHG; (d) peltate trichomes after MHG; (e) and (f) capitate trichomes after HD.

change in the surface tension of the glandular wall, causing it to crumble or rupture more readily. Similar effects have been reported by Pare and Belanger (1997) and by Chen and Spiro (1995) after microwave extraction of rosemary leaves in hexane. When the glands were subjected to more severe thermal stresses and localised high pressures, as in microwave heating, pressure build-up within the glands could have exceeded their capacity for expansion and caused their rupture more rapidly than in conventional extraction.

3.7. Cost, cleanliness, scale-up, and safety considerations

The reduced cost of extraction is clearly advantageous for the proposed MHG method in terms of time and energy. Hydrodistillation required an extraction time of 90 min for heating of 6 l water and 500 g aromatic plants to the extraction temperature, followed by evaporation of the water and essential oil for 180 min. The MHG method required irradiation for 5 min only and hydrodiffusion for 10 min of the *in situ* water and essential oil of the same material. The energy required to perform the extractions is 4.33 kW h for HD, and 0.25 kW h for MHG. The power consumption was determined with a Wattmeter at the microwave generator supply and the electrical heater power supply. With regard to environmental impact, the calculated quantity of carbon dioxide emitted to the atmosphere is greater for HD (3464 g CO_2/g^{-1} essential oil) than for MHG (200 g CO_2/g^{-1} essential oil). These calculations were based on the assumption that to obtain 1 kW h by combustion of fossil fuel 800 g CO_2 will be emitted to the atmosphere (Bernard, 2001) MHG is proposed as an "environmentally friendly" extraction method suitable for sample preparation before essential oil analysis. MHG is a very clean method which avoids the use of large quantities of water and voluminous extraction vessels, in contrast with HD.

MHG could also be used to produce larger quantities of essential oils by using existing large-scale microwave extraction reactors. These microwave reactors are suitable for the extraction of 10, 20, or 100 kg of fresh plant material per batch. These reactors could be easily modified and used for MHG isolations (Pare & Belanger, 1997).

The microwave extraction process is simple and can be readily understood in terms of the operating steps to be performed. Application of microwave energy can pose serious hazards in inexperienced hands, however. High levels of safety and attention to detail must be exercised by all persons planning and performing experiments involving microwaves. Personnel must ensure they

seek proper information from knowledgeable sources and not attempt to implement this technique unless proper guidance is provided. Only approved equipment and scientifically sound procedures should be used at all the times.

3.8. Proposed MHG mechanism

In conventional solvent extraction (Fig. 4), mass transfer occurs from the inside to the outside whilst heat transfer occurs from the outside to the inside. For microwave solvent extraction, the two transport phenomena are in the same direction from the inside of the extracted material to the bulk solvent. The acceleration of extraction rates under microwaves could be due to a synergy combination of the two transfer phenomena mass and heat acting in the same direction. In Microwave Hydrodiffusion and Gravity, heat is dissipated volumetrically inside the irradiated medium, whilst in conventional hydrodistillation, heat is transferred from the heating medium to the interior of the sample. Microwaves are volumetrically distributed, and heat transfers occur from the sample to the colder environment. This causes an important difference between conventional and microwave heating. In conventional heating, heat transfer depends on thermal conductivity, on the temperature difference across the sample, and for fluids, on convection currents. As a result, the temperature increase is often rather slow. By contrast, in microwave heating, due to the volumetric heating effect, much faster temperature increases can be obtained, depending on the microwave power and the dielectric loss factor of the material being irradiated.

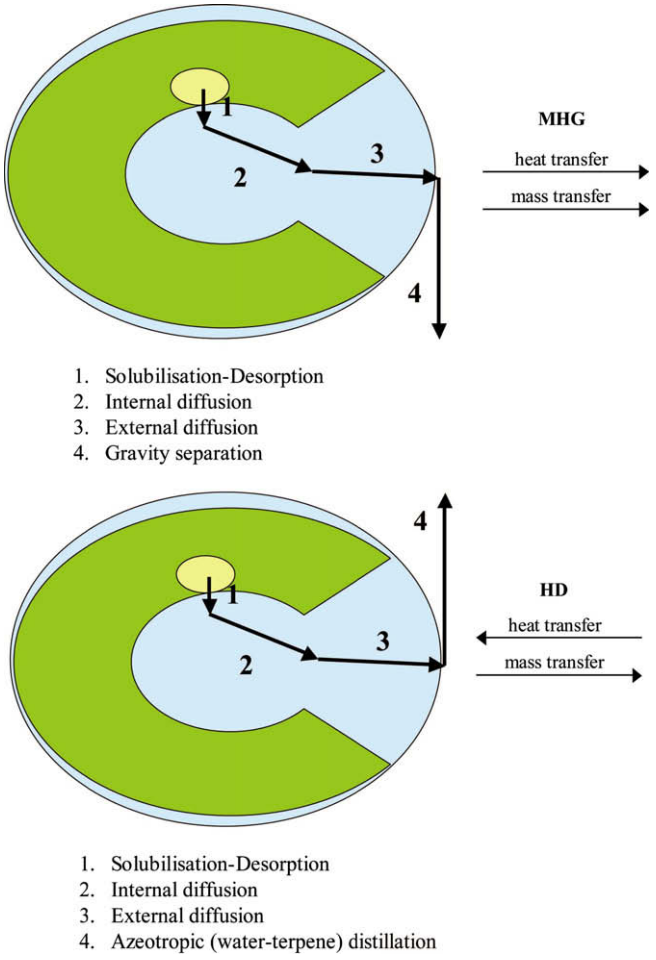

Fig. 4. Schematic representation of the individual steps in the isolation process of essential oils from plant materials.

The acceleration of extraction rates under microwaves could be due to a synergy combination of the two transfer phenomena mass and heat acting in the same direction which are conventionally in opposite for hydrodistillation. Since evaporation is very limited, essential oil and *in situ* water are extracted by this physical phenomenon, known as hydrodiffusion, allows the extract diffused outside the plant material to drop by earth gravity out of the microwave reactor and separated in a vessel traditionally called the "Florentine flask".

4. Conclusion

Microwave extraction makes use of physical and chemical phenomena that are fundamentally different compared with those applied in conventional isolation techniques. This novel process can produce essential oil in concentrate form, free from any residual solvents, contaminants, or artefacts. The new systems developed to date indicate that microwave isolation offers net advantages in term of yield and selectivity, with better isolation time, essential oil composition, and is environmentally friendly.

In this article we have discussed how microwave isolation highly accelerated the isolation process, without causing considerable changes in the volatile oil composition and properties, phenomenon which was already described by Pare and Belanger (1997). The Microwave Hydrodiffusion and Gravity (MHG) of essential oil using fresh *R.* officinalis leaves offers important advantages over traditional alternatives, namely; shorter isolation times; antimicrobial activity more pronounced against yeasts and Gram-positive organisms than against Gram-negative bacteria, environmentally friendly, reduced cost and energy consuming; cleaner features (as no residue generation and no water or solvent used); and the possibility for a better reproduction of natural aroma of the rosemary essential oil than the hydrodistilled essential oil.

Acknowledgements

Authors gratefully acknowledge Cyril Ginoux (Université d'Avignon) for technical support and Isabelle Bornard (INRA – Unité de Pathologie Végétale, Domaine St. Maurice, Montfavet, France) for Electron micrographs.

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