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SOLVENT-FREE MICROWAVE EXTRACTION AS THE USEFUL TOOL FOR EXTRACTION OF EDIBLE ESSENTIAL OILS

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Received: October 15, 2015 / Revised: November 20, 2015 / Accepted: December 12, 2015

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Abstract. Solvent-free microwave extraction (SFME) is proposed as a method for “green” extraction of edible essential oils extensively used in the fragrance, flavour, and pharmaceutical industries and also in aromatherapy. It is a combination of microwave heating and dry distillation, performed at atmospheric pressure without adding any solvent or water. Isolation and concentration of volatile compounds are performed by a single stage. Basil (*Ocimum basilicum* L.) was extracted with SFME at atmospheric pressure and 373 K for 30 min. The extracted compounds were removed from the aqueous extract by simple decantation, identified by gas chromatography–mass spectrometry (GC-MS). Hydrodistillation (HD) of basil was performed with 400 ml of water for 4.5 h for comparison of the results with those provided by the proposed method. SFME method offers important advantages over HD, *viz.* shorter extraction times (30 min *vs.* 4.5 h); better yields (0.13 % *vs.* 0.11 %); environmental impact (energy cost is appreciably higher for performing HD than that required for rapid SFME), cleaner features (as no residue generation and no water or solvent used); and provides a more valuable essential oil. SFME is a green technology and appears as a good alternative for the extraction of edible essential oils from aromatic plants used in aromatherapy and food industry.

Keywords: essential oil, extraction, hydrodistillation, *Ocimum basilicum* L., solvent-free microwave extraction.

1. Introduction

Indonesia has numerous and varied natural resources which are still cannot be used optimally. Among the biodiversity, plants that produce an essential oil, are one of the natural resources which had not fully utilized. Indonesia produces 40-50 kinds of plant essential oil

traded in the world and only some of the types of essential oils are entering the world market, including patchouli, lemongrass, cloves, jasmine, ylang, eucalyptus, sandalwood and vetiver.

One of the essential oil producing plants in Indonesia is not fully utilized, namely basil (*Ocimum basilicum* L.). Indonesian society is currently more popular to consume basil as fresh vegetables at mealtimes by eating or chewing directly fresh basil, whereas basil (*Ocimum basilicum* L.) has many benefits if it will be processed. As a traditional medicine, basil is used to treat fever and nausea [1]. Another use: this plant is often used as an aroma enhancer in foods [2].

Basil leaves contain tannins (4.6 %), flavonoids, steroids, triterpenoids, essential oil (2 %), hexuronic acid, pentose, xylose, molludistin and ursolic acid [3]. Components of essential oil of basil (*Ocimum basilicum* L.) consist of α -pinene, β -pinene, sabinene, myrcene, limonene, 1,8-cineole, *Z*- β -ocimene, *E*- β -ocimene, *E*-sabinene hydrate, *E*- α -bergamotene, β -caryophyllene, *E*- β -farnesene, α -humulene, methyl chavicol, α -terpineol, germacrene-D, β -bisabolene, α -bisabolene, eugenol (62 %), methyl eugenol, α -bisabolol, eucalyptol, estragole, borneol, osimen, geraniol, anethole, α -cadinol, α -terpineol, camphor, 3-octanone, safrole, sesquithujene and linalool [4, 5].

Phytochemical content causes basil leaves to have a variety of properties, including: antimicrobial, expectorant, analgesic, antiasthmatic, antiemetic, diaphoretic, antifertility, hepatoprotective, hypotensive, hypolipidmic, antianaphylactic, antihistaminic, mast cell stabilization, wound healing, radio-protective, antidiabetic, antige-notoxic, antioxidant, anti-carcinogenic, immunologic effect, anticonvulsant, anthelmintic effect, anti-uloerogenic, cardio-protective, and antistress agents [6]. Basil also has a variety of biological and pharmacological effects on the content of essential oil and ethanol extract

of basil leaves which are able to inhibit the growth of bacteria such as *Staphylococcus aureus* and *Escherichia coli* [4].

The development of new separation techniques for the food industries has received a lot of attention lately due to the environmental restrictions, reducing waste water, and the need for minimizing the energy costs. There is also a constant demand to improve the quality of essential oils because consumers demand this quality in their food, pharmaceutical or perfumery products. Essential oils are complex mixtures of volatile substances generally present at low concentrations. Various methods can be used for their extraction, e.g. hydrodistillation, steam distillation and cold pressing. The conventional methods for the extraction of essential oils have some disadvantages. Losses of some volatile compounds, low extraction efficiency, degradation of unsaturated or ester compounds through thermal or hydrolytic effects and toxic solvent residue in the extract may be encountered using these extraction methods. For steam distillation and hydrodistillation, the elevated temperatures for long extraction periods can cause chemical modification of oil components and often a loss of the most volatile molecules. When using solvent extraction, it is impossible to obtain a solvent-free product and this process usually results in the loss of highly volatile components. These shortcomings have led to the consideration of the use of new "green" techniques in essential oil extraction, which typically use less energy and avoid solvents, such as supercritical fluids [7].

The solvent-free microwave extraction (SFME) apparatus is an original combination of microwave heating and dry distillation at atmospheric pressure. SFME was conceived for laboratory scale applications in the extraction of essential oils from different kinds of aromatic plants. Based on a relatively simple principle, this method involves placing plant material in a microwave reactor, without any added solvent or water. The internal heating of the *in situ* water within the "fresh" plant material distends the plant cells and leads to rupture of the glands and oleiferous receptacles. This process thus frees essential oil which is evaporated by *in situ* water of the plant material. A cooling system outside the microwave oven condensed the distillate continuously. The excess of water was refluxed to the extraction vessel in order to restore *in situ* water to the plant material. SFME is neither a modified microwave-assisted extraction (MAE) which uses organic solvents, nor a modified hydro-distillation which uses a large quantity of water [8].

In this paper, the potential of the SFME technique has been compared with a conventional method, hydrodistillation (HD), as the current technique and commercial situation call for research of new extracts and new extraction techniques. We have applied SFME and

HD techniques to extract essential oils from basil (*Ocimum basilicum* L.), which are largely used in food preparation, perfumery and medicine. Natural antioxidants such as those present in basil essential oil may be an alternative source for compounds capable of protecting lipids in foods. Basil essential oil is also used as an antibacterial and antifungal agent. We make appropriate comparisons in terms of extraction yields and rates, essential oil composition, electric consumption and environmental considerations.

2. Experimental

2.1. Plants Material

Basil (*Ocimum basilicum* L.) was collected in Surabaya, East Java, Indonesia. Only fresh plant material was employed in all extractions.

2.2. SFME Apparatus and Procedure

In employing SFME, we used a domestic microwave oven (EMM-2007X, Electrolux, 20 l, maximum delivered power of 800 W) with wave frequency of 2450 MHz. The dimensions of the PTFE-coated cavity of the microwave oven were 46.1x28.0x37.3 cm. The microwave oven was modified by drilling a hole at the top. A round bottom flask with a capacity of 1000 ml was placed inside the oven and was connected to the three-way adapter and Liebig condenser through the hole. Then, the hole was closed with PTFE to prevent any loss of the heat inside.

In a typical SFME procedure performed at atmospheric pressure, 150 g of fresh plant materials was placed inside the reaction flask and heated by microwave irradiation with 400 W (50% power) for 30 min without added any solvent or water. During the process, the vapor passed through the condenser outside the microwave cavity where it was condensed. Essential oil and water were simply separated by decantation. The essential oil was collected in amber vials, dried under anhydrous sodium sulfate and stored at 277 K. The extraction yield of EO was calculated according to the given equation:

$$\text{Extraction yield (\%, w/w)} = \frac{\text{Mass of extracted essential oil}}{\text{Mass of fresh material}} \cdot 100$$

2.2. Hydrodistillation Apparatus and Procedure

Hydrodistillation was employed more or less like SFME, but a laboratory hot plate (CORNING PC-600, 1043 W, 50 Hz) was used instead of the microwave oven. One hundred and fifty grams of fresh plant material and 400 ml of distilled water (plant material-to-water ratio is

0.375) were placed into HD with a Liebig condenser, and EOs were extracted for 4.5 h (until no more essential oil was obtained). EO was collected in amber vials, dried under anhydrous sodium sulfate and stored at 277 K.

2.3. Chemical Analysis of Essential Oil Constituents

Essential oils composition was determined by gas chromatography coupled to mass spectrometry (GC-MS) analysis on a Hewlett-Packard 6890 gas chromatograph coupled to 5973A mass spectrometer, using two fused-silica-capillary columns with different stationary phases. The non-polar column was HP5MS™ (30 m length, 0.25 mm diameter and 0.25 μm film thickness) and the polar one was Stabilwax™ consisting of Carbowax™-PEG (60 m length, 0.25 mm diameter and 0.25 μm film

thickness). GC-MS spectra were obtained using the following conditions: carrier gas He; flow rate 1.0 ml·min⁻¹; split 1:50; injection volume 1.0 μl; injection temperature 573 K; oven temperature progress from 373 to 523 K at 10 K/min; the ionisation mode used was electronic impact at 70 eV. Most constituents were tentatively identified by comparison of their GC Kovats retention indices (RI), determined with reference to the homologous series of C₅–C₂₈ *n*-alkanes and with those of authentic standards available in the authors' laboratory. Identification was confirmed by comparison of their mass spectral fragmentation patterns with those stored in the MS database (National Institute of Standards and Technology and Wiley libraries) and with mass spectra literature data [9, 10]. 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 a relative amount of that compound.

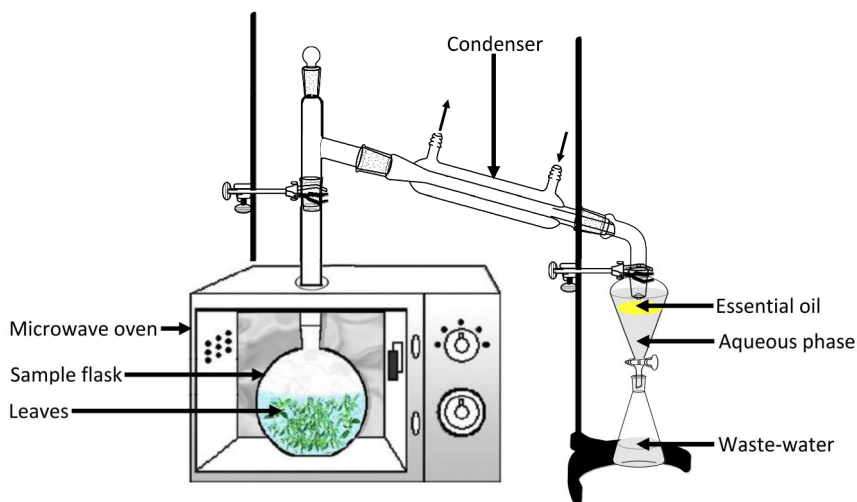


Fig. 1. Schematic representation of the solvent-free microwave extraction apparatus used in this study

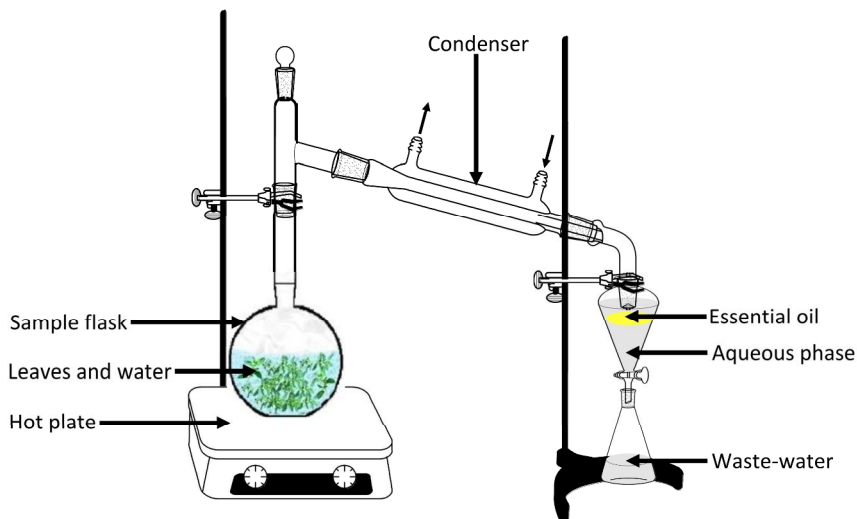


Fig. 2. Schematic representation of the hydro-distillation apparatus used in this study

Table 1

Chemical compositions of essential oils obtained by solvent-free microwave extraction (SFME) and hydrodistillation (HD) of basil (*Ocimum basilicum* L.)

Number	Compound	RI	Essential oil constituents, %	
			HD	SFME
1	α -Pinene	935	0.70	0.00
2	Sabinene	973	0.04	0.00
3	Octen 3 ol	977	1.22	0.03
4	β -Pinene	978	0.25	0.05
5	Heptanol	987	0.64	0.00
6	β -Myrcene	991	0.37	0.00
7	<i>p</i> -Cymene	1026	0.00	0.04
8	Limonene	1027	0.41	0.16
9	1,8-Cineole	1030	0.19	0.28
10	β -Ocimene <E>	1046	0.00	0.19
11	γ -Terpinene	1056	0.00	0.04
12	Fenchone	1087	1.09	0.09
13	Linalool	1098	0.31	0.58
14	Camphor	1141	0.00	0.06
15	Menthol	1169	0.00	0.16
16	α -Terpineol	1189	0.00	0.09
17	Methyl chavicol	1201	37.63	13.85
18	Nerol	1227	1.14	0.75
19	Neral	1242	12.61	4.14
20	Geraniol	1253	0.63	0.85
21	Geranial	1272	14.19	5.73
22	α -Terpinenyl acetate	1343	0.00	0.14
23	Neryl acetate	1360	0.00	0.11
24	α -Copaene	1370	0.42	0.81
25	Geranyl acetate	1378	0.00	0.69
26	β -Bourbonene	1379	0.22	0.00
27	β -Cubebene	1384	0.49	0.94
28	β -Elemene	1386	0.00	0.60
29	Methyl eugenol	1399	1.02	1.05
30	β -Caryophyllene	1407	5.01	14.86
31	α -Bergamotene <Z>	1430	1.15	0.00
32	α -Humulene	1446	4.04	13.59
33	β -Farnesene <Z>	1451	0.21	1.56
34	Germacrene-D	1474	1.21	8.68
35	γ -Cadinene	1507	0.00	0.47
36	Δ -Cadinene	1516	0.00	0.47
37	α -Bisabolene <E>	1538	1.54	9.45
38	β -Bisabolene	1538	0.00	0.60
39	Spathulenol	1569	0.21	0.00
40	Caryophyllene oxide	1574	2.58	1.51
41	α -Cadinol <Epi->	1633	0.00	2.38
Total peak area, %			89.52	85
Total extraction time, min			270	30
Yield, %			0.11	0.13

2.4. CO₂ Emissions

The measurements of CO₂ emitted were carried out based on the procedures mentioned in the previous studies: to obtain 1 kWh of energy from coal or fossil fuels, 800 g of CO₂ will be released into the atmosphere during their combustion [11].

3. Results and Discussion

3.1. Extraction Yield and Time

One of the advantages of the SFME method is rapidity. The extraction temperature is equal to the boiling point of water at atmospheric pressure (373 K) for both the SFME and HD extraction methods. To reach the extraction temperature (373 K) and thus obtain the first essential oil droplet, it is necessary to heat for only 2 min with MAD compared with 50 min for HD. As shown in Table 1, the ultimate yield of essential oils extracted by SFME after 30 min of operation was greater than that obtained by HD after 4.5 h of operation (0.13 % vs. 0.11 % w/w). These results mean a substantial saving in time.

3.2. Composition of Essential Oil

The identities of the extracted essential oils from both methods are shown in Table 1. A total of 41 compounds were identified in basil essential oil using two techniques. Using SFME, 34 compounds were detected, while 27 compounds were detected in HD. SFME and HD enabled the detection of the most volatile active compounds in basil essential oil, such as methyl chavicol, neral, geranial, β -caryophyllene, α -humulene, and α -bisabolene, but their proportions depend strongly on the extraction technique. Methyl chavicol, a oxygenated monoterpene was present at 37.63 and 13.85 %, respectively, for HD and SFME. Neral, a oxygenated monoterpene was present at 12.61 and 4.14 %, respectively, for HD and SFME. Geranial, a oxygenated monoterpene was present at 14.19 and 5.73 %, respectively, for HD and SFME. β -Caryophyllene, a sesquiterpene hydrocarbon was present at 5.01 and 14.86 %, respectively, for HD and SFME. α -Humulene, a sesquiterpene hydrocarbon was present at 4.04 and 13.59 %, respectively, for HD and SFME. α -Bisabolene, a sesquiterpene hydrocarbon was present at 1.54 and 9.45 %, respectively, for HD and SFME.

Some more new compounds were found as a result of SFME but their number and amounts are very small. The loss of some compounds in SFME compared with HD is probably not due to lack of their extraction but rather that the reduction in extraction time and the amount

of water in the SFME method reduce the degradation of compounds by hydrolysis, trans-esterification or oxidation, and hence there are fewer degradation products noted in the analysis.

3.3. Electric Consumption and Environmental Considerations

The reduced extraction time is clearly advantageous for the proposed SFME methods in terms of cost and energy. The energy requirement needed to perform the extraction methods, based on the power consumptions of the hot plate (in HD) and the microwave oven (in SFME), considering the total periods of full extractions, was 4.6935 kWh for HD and 0.2000 kWh for SFME (Fig. 3). Li *et al.* [12] showed that for the energy requirements, SFME needs less than 0.5 kWh for normal performance but conventional methods expend more than 4.5 kWh. Relative electric consumption for the production of 1 g EO in HD and SFME was 29.3344 and 0.9452 kWh/g EO, respectively (Fig. 3). This indicates a substantial saving in the extraction cost when using SFME instead of HD. Filly *et al.* [13] reported that the energy required for the extraction of EOs from rosemary was 4.50 kWh per gram of EO in HD, but 0.25 kWh per gram of EO in SFME.

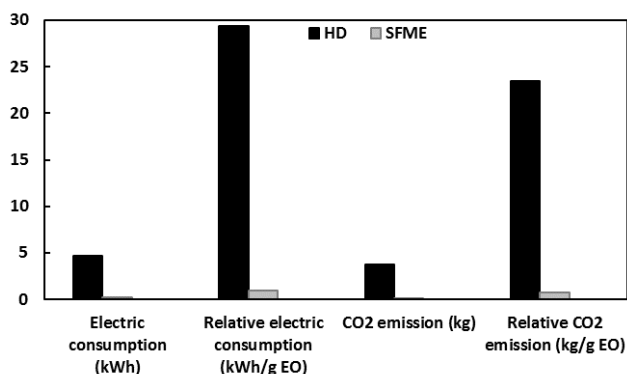


Fig. 3. Electric consumption of hydrodistillation (HD) and solvent-free microwave extraction (SFME) of essential oil (EO) from basil (*Ocimum basilicum* L)

Regarding the environmental impact of pollution, the calculated quantity of CO₂ emitted in the atmosphere was higher in the case of HD (3.7548 kg CO₂) than those of SFME (0.1600 kg CO₂) (Fig. 3). Relative amounts of CO₂ emissions that result from the production of 1 g EO were higher in HD (23.4675 kg CO₂/g EO) than those in SFME (0.7561 kg CO₂/g EO) (Fig. 3). This finding further indicated that there was a significant difference between SFME and HD in terms of the amount of CO₂ released into the atmosphere for the production of 1 g EO.

Also, waste water was lower in SFME than HD. Filly *et al.* [13] showed that the amount of CO₂ released into the atmosphere was dramatically higher in HD (3600 g CO₂/g EO) than that in SFME (200 g CO₂/g EO). Also, Li *et al.* [12] reported that the quantity of CO₂ calculated to be rejected in the atmosphere is higher in the case of HD (3600 g CO₂/g EO) than for SFME (200 g CO₂/g EO).

4. Conclusions

The solvent-free microwave extraction (SFME) method offers important advantages over traditional hydrodistillation. It is quicker, more effective and has an environmentally friendly approach. These reasons make SFME a promising tool for the extraction of essential oils from aromatic and medicinal plants which is of great concern in food industry and aromatherapy.

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ЗАСТОСУВАННЯ МІКРОХВИЛЬОВОЇ ЕКСТРАКЦІЇ БЕЗ РОЗЧИННИКА ДЛЯ ЕКСТРАКЦІЇ ХАРЧОВИХ ЕФІРНИХ ОЛІЙ

Анотація. Запропоновано використовувати мікрохвильову екстракцію без розчинника (МЕБР) як метод «зеленої технології» для екстракції харчових ефірних олій, які широко використовуються у парфумерній та фармацевтичній промисловості, а також в ароматерапії. Такий метод є поєднанням мікрохвильового нагріву та сухої перегонки за атмосферного тиску без додавання будь-якого розчинника або води. Виділення і концентрування летких сполук відбувається одностадійно. За допомогою вказаного методу проведено екстракцію базилику (*Ocimum Basilicum L.*) за атмосферного тиску і 373 K протягом 30 хв. Екстраговані компоненти видалені з водного екстракту простою декантацією та ідентифіковані за допомогою газової хроматографії-мас-спектрометрії (ГХ-МС). Для порівняння отриманих результатів проведено гідродистиляцію (ГД) базилику з 400 мл води протягом 4,5 год. Доведено, що запропонований МЕБР метод має важливі переваги порівняно з ГД, а саме: скорочення часу екстракції (30 хв проти 4,5 ч); вищий вихід (0,13% проти 0,11%); менший вплив на навколишнє середовище (затрати енергії є значно нижчими), не використовується вода або розчинник і не утворюються залишки; отримується більш цінна ефірна олія. Показано, що метод МЕБР відноситься до «зелених технологій» і є хорошою альтернативою для вилучення харчових ефірних олій з ароматичних рослин, які використовуються в ароматерапії і харчовій промисловості.

Ключові слова: ефірна олія, екстракція, гідродистиляція, *Ocimum Basilicum L.*, мікрохвильова екстракція без розчинника.