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Microwave Assisted-Extraction of Essential Oil from Fresh Basil (*Ocium basilicium* L.) Leaves

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Abstract. In this research, the extraction of essential oil from fresh basil leaves using solvent-free microwave extraction (SFME) and microwave hydrodistillation (MHD) methods. Several parameters influence the extraction of basil oil using the SFME method: microwave power, the ratio between the mass of raw material with a volume of distiller (F/D), material size, and length of extraction time. Besides, the components contained in basil oil and changes in oil gland conditions in basil leaves before and after being extracted were also evaluated. The optimum condition was obtained as follows: microwave power of 380 W, the ratio between the mass of raw material with a volume of distiller (F/D) of 0.1 g/mL, raw material size of intact (± 3 cm), with an extraction time of 60 min. Moreover, SFME has a shorter extraction time to produce yields than MHD methods. GC-MS analyzed the composition of basil oil, and there were 49 identified components. This study shows that the SFME method is more effective than the MHD method for the extraction of basil oil from fresh leaves based on time extraction and yield.

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

1. Introduction

Essential oil is one of the potential agroindustry export commodities as a mainstay for Indonesian foreign exchange. The data on essential oil consumption and its derivation from World export-import statistics shows an average growth of 5-10% per year. The increase was mainly driven by the growing need for cosmetics, food flavoring, and fragrance industries.

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Essential oils that are mostly distilled in Indonesia include patchouli oil, cloves, nutmeg, citronella, fragrant root, eucalyptus oil, and others. Meanwhile, essential oils have the potential to be developed, including basil, gandapura, cardamom, cinnamon, and others [1].

Basil (*Ocimum basilicum* L.) is one of the essential oil-producing plants in Indonesia that has not been fully utilized. Currently, Indonesians are more likely to consume basil leaves as a vegetable (*Ocimum basilicum* L.). As a traditional medicine, basil leaves are used to treat fever, nausea, and increase breast milk production [2]. Besides, the other benefits of basil are to heal various diseases, such as headaches, cough, diarrhea, constipation, skin diseases, worm diseases, and kidney failure. As a medicine, basil leaves are also efficacious among others in anti-carcinogenic, anthelmintic, antiseptic, anti-rheumatic, anti-stress, and antibacterial [3]. Basil leaves contain various components, including saponins, flavonoids, tannins, and essential oils [4]. While the most important content is an essential oil, the essential oils in basil leaves can inhibit the growth of *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Pseudomonas fluorescen*ce, *Candida albicans*, *Streptococcus alfa,* and *Bacillus subtilis* [5].

In a previous study, essential oil from basil leaves was obtained from dry basil by using the soxhlation method. Basil leaves were extracted using the soxhlation method and ethyl acetate as a solvent. In this study, basil leaves were dried and sieved in 40 mesh. The optimal condition from this study was a 1:6 (w/v) ratio of dry basil leaves and solvent for 6 hours of extraction time [6]. Another method that was used is the microwave hydrodistillation (MHD) method. Based on a study conducted by Dalia et al. (2015), using 200 g of dried basil leaves using the MHD method for 6 h obtained a yield of 0.6% v/w. The MHD method obtained a small yield and the length time of extraction, it is necessary to consider using the green technique in the extraction essential oil method with minimum solvent, energy, and time. Nowadays, Microwave-assisted extraction is one of the new methods that have been developed for essential oil extraction. A study comparing MHD and the Solvent-Free Microwave Extraction (SFME) method to extract essential oils from basil leaves was done. From this study, dry basil leaves were extracted to obtain essential oils. From this study, SFME has significant advantages over MHD, such as extraction time, solvent saving, substantial energy, and being environmentally friendly [7]. From the research, we can conclude that all the studies use dry basil leaves to obtain essential oil, and SFME is the optimal method for essential oil extraction.

This study aims to optimize the process of extracting the essential oil from fresh basil leaves. The selection of fresh basil leaves had not been used in previous studies and was expected to reduce costs due to no drying process required. This study will use two methods of microwave-assisted extraction. These methods are the MHD method and the SFME method. The selection of the SFME method to extract essential oils does not require the addition of solvent as other extraction methods, higher yield, and shorter extraction time [8].

2. Materials and Methods

2.1 Materials

Fresh basil (*Ocimum basilicum* L.) leaves were collected from Keputran market, Surabaya, East Java, Indonesia. The size of fresh basil leaves used in this study was intact (± 3 cm), half intact (± 1.5 cm), and chopped (± 0.5 cm). An analytical grade of anhydrous sodium sulfate was purchased from Sigma-Aldrich, Singapore.

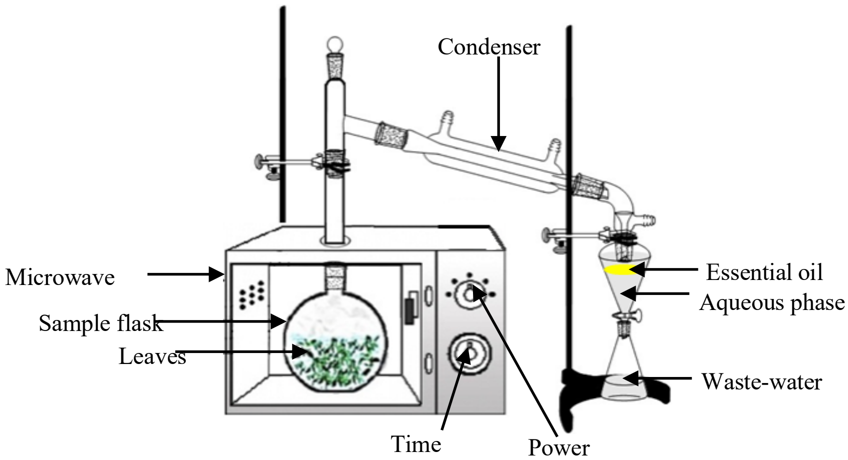

Figure 1. The experimental setup for extraction of essential oil from fresh basil (*Ocimum basilicum* L.) leaves using a solvent-free microwave extraction method

In this SFME method (Figure 1), we used a microwave oven type EMM-2007X, 20 L, 220 V, maximum power of 800 W) with a wave frequency of 2450 MHz. The dimensions of the PTFE-coated cavity of the microwave oven were 46.1 x 28 x 37.3 cm. The microwave oven was modified by drilling a hole at the top. A round bottom flask capacity of 1000 mL was placed in the oven and connected to the Clevenger apparatus. Then, the hole was closed with PTFE to prevent any loss of heat inside.

The procedure for the SFME method was performed at 1 atm, the sizes variables of fresh basil leaves (chopped, intact, and half intact) and the ratio between the mass of raw material with a volume of distiller (F/D) (0.1; 0.175; and 0.25 g/mL)—the weighted material placed in a flask (1000 mL). Four power levels were operated using a microwave oven (100 W, 240 W, 380 W, and 540 W) for 60 min. Furthermore, to find out the effect of extraction time on the yield of basil oil, extraction using the SFME method was done up to 80 min. The extracted basil oil was dried over anhydrous sodium sulfate to remove water in the essential oil. Then weighed and stored in a vial bottle at 4 °C.

The yield of basil oil calculation is as follows:

$$\text{Yield (\%, w/w)} = \frac{Mass\ of\ extracted\ basil\ oil}{Mass\ of\ fresh\ basil\ leaves \times (1 - water\ content)} \times 100\% \tag{1}$$

2.2 Microwave Hydrodistilattion (MHD)

The extraction of basil oil using the MHD method (Figure 2) is done as the SFME method. 175 g intact fresh basil leaves (± 3 cm) and 400 mL of distilled water (ratio of raw material and a volume of solvent (F/S) of 0.4375 g/mL) were put into a 1000 mL distiller flask and basil oil extracted on microwave power of 380 W for 180 min. Basil oil was collected in a vial bottle, then dried over anhydrous sodium sulfate and stored at 4 °C.

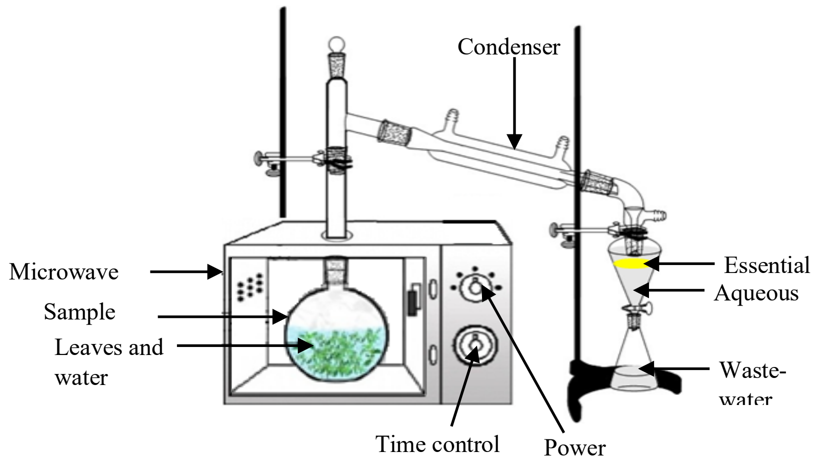

Figure 2. The experimental setup for extraction of essential oil from fresh basil (*Ocimum basilicum* L.) leaves using the microwave hydrodistillation method

2.3 Chemical analysis of basil oil components by gas chromatography-mass spectrometry (GC-MS)

The components contained in basil oil were obtained by using gas chromatography-mass spectrometry (GC-MS) analysis. This analysis is not only used to know the components contained in essential oils, but also to know the level of each component. GC-MS was used in this study using an Agilent 6980N gas chromatograph with an Agilent 5973 mass spectrometric detector. Gas chromatography-mass spectrometry was incorporated with chromatography column HP-5.5% phenyl methyl siloxane, 30 m length; 0.32 mm film thickness, 0.25 µm internal diameter.

2.4 Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) analysis is a test used to analyze the material's surface structure. The fresh basil leaf morphology was performed using SEM Evo MA 19. In this case, the material analyzed was the basil leaves before and after extraction.

3. Results and Discussions

3.1 Effect of microwave power on yield of basil oil comparison of performance basil extraction using SFME and MHD

The time of extraction is the main factor that affects the performance of MHD and SFME. In the extraction process, there are three important stages: the equilibrium phase, the transition phase, and the diffusion phase. The equilibrium phase occurs when the substrate is transferred to the outer layer of the matrix. Then, it is followed by the transition phase in which convection and diffusion occur in mass transfer. The last phase is diffusion, in the diffusion phase, the release of extracts through a diffusion mechanism is characterized, and in this phase, the extraction rate is slow. In the extraction process, mostly extraction time is proportional to yield. The longer the extraction time, the higher yield will be obtained [9].

In extracting basil oil using the MHD method and SFME method, the time of extraction is one of the factors that need to be considered. Table 1 shows the correlation between extraction time and basil oil yield. The MHD method requires more time to reach the equilibrium phase than the SFME method, which is 80 min. Whereas in the SFME method, within 80 min, the diffusion phase has reached. In general, the extraction of basil oil using the SFME method required a faster time (60 min) that could produce higher yields, which is 3.41 times greater than the MHD method. In SFME, extraction occurs in two kinds of heating: selective and volumetric. Selective heating is microwave radiation that can directly penetrate the distillation flask so that materials and solvents can absorb radiation effectively. Volumetric heating is heating that occurs in the overall volume of material so that heat is uniform and occurs more quickly. This method allows to obtain a high yield at a lower extraction time when the extraction was done by SFME [11]. This statement proves that the SFME method is quite effective and efficient when compared to the MHD method.

Table 1. Comparison of the effect of time on yield between SFME and MAHD

Time (minutes)	Yield (%)	
	SFME	MAHD
0	0	0
20	1.662	0.249
40	2.120	0.268
60	2.281	0.388
80	2.373	0.400
100		0.503
120		0.573
140		0.603
160		0.666
180		0.668

3.2 Effect of operation condition on basil oil yield using SFME

Power in the microwave extraction process is an important operating condition that affects the extraction yield. In the extraction process, the amount of energy received by the raw material is converted into heat energy by microwave power, and the heat energy will help the process of removing essential oils from the raw material [10].

In basil leaf oil extraction, determining the optimal power is important because it will affect the temperature during the extraction process. Figure 3 shows the effect of microwave power on temperature. The higher the microwave power, the more polar molecules of the material will have faster rotation to produce heat energy, which is detected from an increase in temperature. The temperature-time profile for each power used in the extraction process is in Figure 3. The slope of the linear line determines the rate of the temperature rising at the profile temperature. From Figure 3, the temperature increase for each power used in the SFME method is 3.35 °C/min; 14.48 °C/min; 18,7 °C/min; and 23 °C/min for 100 W, 240 W, 380 W, and 540 W microwave power respectively.

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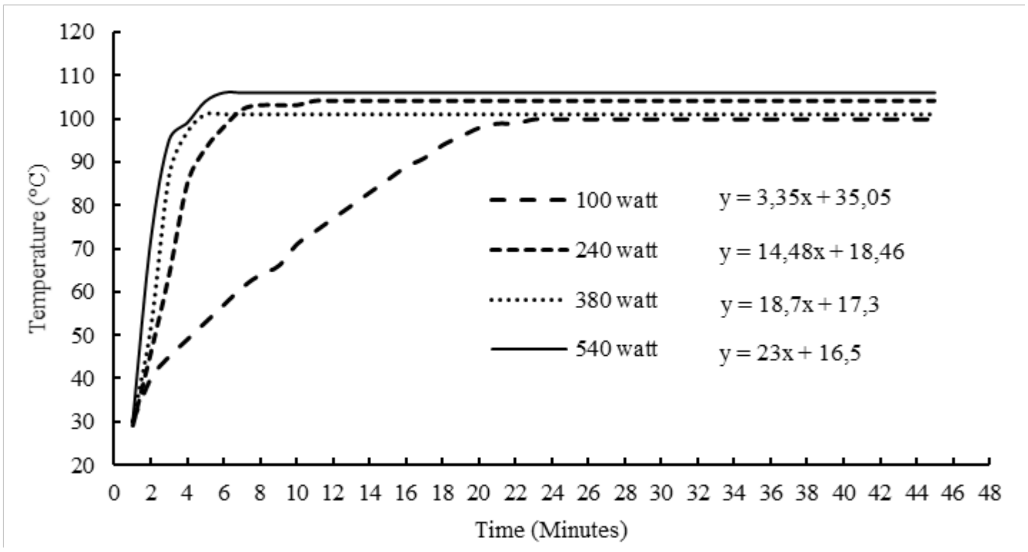
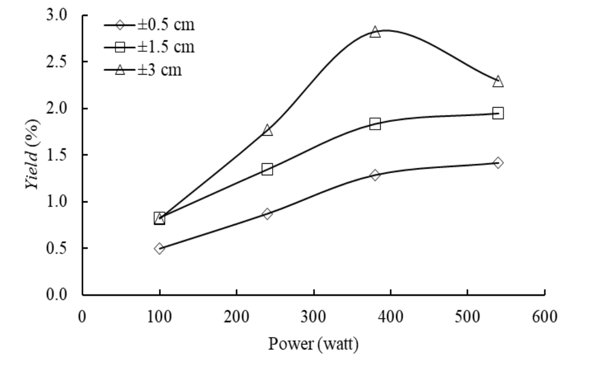
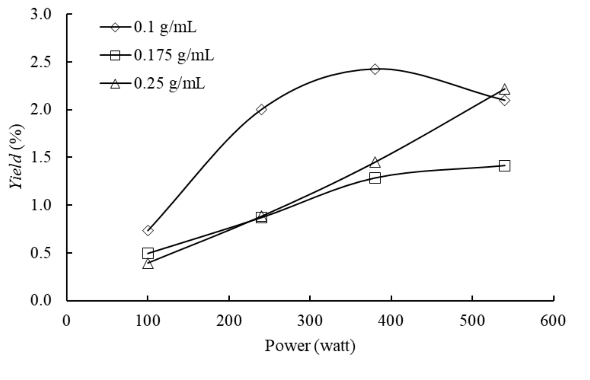


Figure 3. Temperature-time profile for each microwave power

Figure 3 shows the microwave power of 540 W, which resulted in the fastest increase in temperature. However, in the extraction process using SFME methods, there is an essential factor of the material that affects the extraction process when using power 540 W, not necessarily the yield produced was the most exceptional. In this study, the microwave power that provided the highest yield was at 380 W.



(a)



(b)

Figure 4. Effect of microwave power on basil oil yield at (a) ratio 0.175 g/ml and (b) size ± 0.5 cm

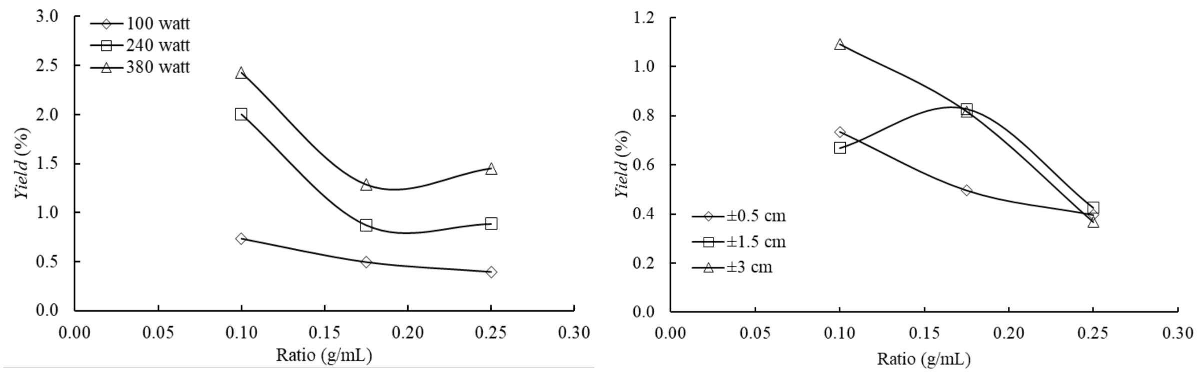
Based on Figure 4 (a), there was a tendency to increase the yield as power increases. Thus, the amount of energy received by the material converted into heat would generate a higher yield of basil oil. Microwaves can accelerate the extraction process for the desorption of compounds targeted from the matrix with low and high power [11]. However, in the leaf intact (± 3 cm), the oil yield basil was decreased when the power increased from 380 W to 540 W.

This is because the ratio of the material space in the distillation flask to the ratio of material to the size of the whole leaf (± 3 cm) is more than the other flask. According to the previous study, the more full of materials in the flask, the slower the oil extraction rate is since the evaporation process of oil obstructs; thus, it decreases the yield of essential oil [12].

In Figure 4 (b), there is a tendency to increase yield as power increases. However, at the smallest ratio of 0.1 g / mL, the highest yield was obtained at 380 W. It was caused when the microwave power 540 W, by degradation of materials and components of essential oils at the lowest material ratio, resulted in the decreased yield. In this study, the highest yield of extracted basil oil from fresh ingredients by the SFME method was obtained when microwave power was 380 W. This microwave power was the most effective in this study.

3.3 Effect of ratio raw material mass and distiller volume (F/D) on basil oil yield

The mass of material used was 100, 175, and 250 g for each variable size. The mass of this material affected the mass ratio of the material per volume distiller. Figure 5 shows the effect of the ratio of raw material mass and distiller volume (F/D) to basil oil yield studied at the lowest microwave power of 100 W. Low microwave power is expected to minimize the significant effect of microwave heat so that the resulting data is more stable.



(a) (b)

Figure 5. The effect of the ratio on yield basil oil size ±0.5 cm and (b) power 100 W

Based on Figures 5 (a) and (b), the higher the F/D ratio, the lower yield was obtained. The optimum yield for extraction of basil oil with the SFME method was found in the F/D ratio of 0.1 g/mL, it is caused by the smallest ratio of basil extracted with a medium density. The material density factor is the ratio between the mass of the material and the volume capacity of the distiller flask used. The ratio used relates to how much of the raw material enters the distiller

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flask. Thus, the oil extraction and evaporation process can proceed perfectly. The material density that is too high and uneven causes the formation of steam lines "rat holes" which may reduce the yield and quality of essential oils [13]. A previous study by Kusuma (2017) on patchouli oil extraction using the SFME method with several variations in the ratio of the mass of raw materials to volume distillers (F/D) 0.06, 0.08, 0.1 and 0.12 g/mL. The highest yield of these variable ratios was obtained at the lowest ratio of 0.06 g/mL.

3.4 Effect of material size on essential oil yield

In this study, the size of the materials used are intact (± 3 cm), half intact (± 1.5 cm), and chopped (± 0.5 cm). From Figure 5, we can see the effect of the material size of the basil leaf on the extraction yield using the SFME method. Based on Figure 5, the highest yield is in each power, and the ratio was the size of leaves ± 3cm. Whereas, the leaf size ± 1.5cm and ± 0.5 cm yield was getting lower along with the smaller size of the material. This statement is contrary to the previous study. It mentioned that the smaller material size would obtain a higher yield because the small size of the material will accelerate the diffusion process [14]. In this study, the size of the material that produces optimal yield was the largest. In this study, water content takes the effect of the extraction process. The largest leaf size has the most substantial moisture content. Thus, the extraction process cannot be easily scorched and can be extracted optimally. Meanwhile, the size of the materials with a smaller amount of water content quickly burns, or the ingredients cannot be extracted optimally.

The ratio of 0.1 g/mL resulted in the largest yield when the material size was ± 0.5 cm. This ratio is different from other ratios, which are 0.175 and 0.25 g/mL. With the effect of the water content in fresh basil ingredients, it should be the extraction with the smallest ratio of 0.1 g/mL, and the smallest material size of ± 0.5 cm obtained a low yield. The small ratio and size material contained the water slightly. Thus, the material was scorched more quickly and burned, and that condition caused the material not extracted perfectly. However, in this condition, the extraction was carried out at 380 W. Thus, it was possible that the material had been extracted perfectly and produced a large yield.

3.5 Analysis of gas chromatography-mass spectrometry (GC-MS) basil oil

GC-MS analysis was not only used to find out the components contained in essential oils but also used it to determine the levels for each component. As shown in Table 2 on basil oil from fresh basil leaves, the number of components contained in it was 49 components with the highest component content E-Citral 32.771% and Z-Citral 27.618%. From these results, it was confirmed that the type of basil used in this study was the citral type, which is in line with research from Mondello et al. (2001) with components of basil essential oil in the form of geranial (E-Citral) 33.70% and mineral (Z-Citral) 27.90%.

Table 2. Components contained in essential oils of basil leaf (ratio of 0.175 g / ml, size ± 3 cm, and power 380 W) based on GC-MS analysis

No.	R.T. (min)	Compound	% Area
		Monoterpenes	
1	5.565	β-Ocimene	0.596
2	5.624	β-Myrcene	0.123
3	8.176	trans-chrysanthemal	0.651
4	10.449	Camphene	0.133
5	10.832	Bicyclo [3.1.0]hexane, 6-isopropylidene-1-methyl-	0.112
6	14.351	Naphtalene, decahydro-, cis-	0.732
		Oxygenated Monoterpenes	
7	7.201	D- (+) -Fenchone	0.153
8	7.453	Linalool L	2.607
9	8.002	cis/cis-Photocitral	0.184
10	9.761	Z-Citral	27.618
11	9.857	Piperiton	0.123
12	10.919	(+) -Carvotanacetone	0.269
13	11.398	D-Fenchyl alcohol	0.332
14	11.503	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate	0.649
15	11.973	E-ocimenone	0.141
		Sesquiterpenes	
16	10.275	E-Citral	32.771
17	11.102	(Z)-β-Farnesene	0.332
18	11.459	Copaene	0.24
19	11.66	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis (1-methylethenyl)-, [1S-(1α,2β,4β)]-	0.426
20	11.903	α-Gurjunene	0.228
21	12.078	β-Caryophyllene	5.449
22	12.156	1H-Cyclopenta [1,3]cyclopropa [1,2]benzene, 2,3,3aα, 3bα., 4,5,6,7-octahydro-4α -isoprophyl-7β	0.113
23	12.217	α-Bergamotene	2.201
24	12.443	(E)-β-Farnesene	0.497
25	12.487	α-Humulene	1.576
26	12.557	Bicyclo [7.2.0] undec-4-ene, 4,11,11-trimethyl-8-methylene-	0.123

No.	R.T. (min)	Compound	% Area
27	12.739	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1α,4aα.,8aα)-	0.124
28	12.826	Germacrene-D	2.66
29	13.096	(+,-)-β-Bisabolene	0.113
30	13.192	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1α,4aβ,8aα)-	0.116
31	13.297	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	0.69
32	13.532	cis-α-Bisabolene	4.471
33	13.671	2-Pentadecen-4-yne, (Z)-	0.233
		Oxygenated Sesquiterpenes	
34	13.68	Caryophyllene oxide	3.079
35	14.673	d-Nerolidol	0.261
		Other Compounds	
36	8.672	Ethenylcyclohexane	2.074
37	19.393	1,3,5-Cycloheptatriene, 2,4-dihexyl-7, 7-dimethyl-	0.383
		OtherOxygenated Compounds	
38	5.546	Methyl heptanone	0.689
39	8.089	Dicyclopropyl Ketone	0.18
40	8.402	(S,E)-3-Methyl-2-methylene-4 -hexenal	1.601
41	8.551	Rosefuran epoxide	0.12
42	8.908	Methyl chavicol	0.21
43	10.728	2,6-Octadienoic acid, 3,7-dimethyl-, methyl ester	0.17
44	11.242	Neryl acetate	0.712
45	11.538	cis-3-Hexenyl Lactate	0.286
46	16.031	Benzyl benzoate	2.878
47	17.094	Benzyl salicylate	0.268
48	17.999	Geranyl benzoate	0.15
49	19.645	2-Buten-1-one, 1-(2,2,5a-trimethylperhydro-1-benzoxiren-1-yl)	0.154
		Monoterpenes	2.347
		Oxygenated Monoterpenes	32.076
		Sesquiterpenes	52.363
		Oxygenated Sesquiterpenes	3.34
		Other Compounds	2.457
		Other Oxygenated Compounds	7.418

The components contained in essential oils can be classified into several compounds, namely monoterpenes, oxygenated monoterpenes, sesquiterpenes, oxygenated sesquiterpenes, other compounds, and other oxygenated compounds. The oxygenated compound has more

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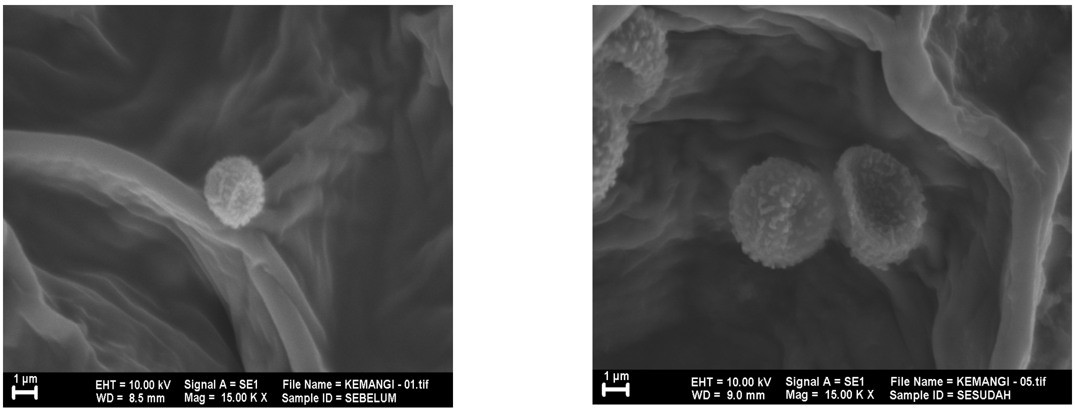
influence on the aroma of essential oils compared to other compounds [15]. In this study, based on the GC-MS test, it was found that the number of oxygenated compounds in this basil oil was 42.834%.

3.6 Scanning electron microscopy

Scanning Electron Microscopy (SEM) is a test used to analyze the surface structure of materials. In this study, the material analyzed was basil leaves before and after extraction. Based on Figure 6, it can be seen that there are still intact oil glands (perfect shape) in the cross-section of basil leaves before being extracted. However, the oil gland extraction process was not complete because the oil inside had been taken. In the sample of basil leaves after extracting in Figure 6 (b), the shape of the oil gland was only slightly concave and not damaged. This was due to the ratio of the material used when the extraction process was a large ratio of 0.25 g / mL, so there was a possibility that the leaves which was used for SEM samples were not perfectly extracted because of the density factor of the material.



(a) (b)

Figure 6. SEM results of fresh basil leaves with a magnification of 15,000 times (a) before extraction and (b) after extraction

4. Conclusion

In this study, the optimal operating conditions using the SFME method were 380 W microwave power, the ratio between the mass of the raw material and the volume distiller (F / D) 0.1 g / mL, the size of whole basil leaves (± 3 cm), and extraction time of 60 min. Based on GC-MS analysis, there are 49 components in basil oil, and the most significant component is in the form of E-Citral. Thus, the basil ingredients used are classified as Citral type. SEM analysis is used to observe changes in the shape of the oil glands found in basil leaves before and after extraction, which shows that the oil has been extracted. The SFME method significantly reduced extraction time and increased extraction yield compared to the MHD method. SFME was 60 min faster than MHD yielding a 3.41-fold higher result. Thus, the SFME method can be applied to extracting basil oil as an effective and efficient method to obtain higher yields.

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