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Effects of pre-treatment with *Aspergillus awamori* and extraction methods on essential oil yield from spearmint leaves (*Mentha spicata* L.)

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ABSTRACT

Extraction of essential oil from spearmint leaves is typically hindered by the presence of cell wall composed of lignocellulose which can be biologically degraded by microorganisms. This study aimed to investigate the effects of fermentation using *Aspergillus awamori* towards the lignocellulosic content of spearmint (*Mentha spicata* L.) leaf as well as spearmint oil yield and composition, and diffusion coefficient obtained using different extraction methods. Fermentation of the spearmint leaves were carried out for 3, 6, and 9 days followed by drying and extraction using three different techniques particularly Soxhlet, hydrodistillation and maceration. After fermentation, the cellulose, hemicellulose, and lignin reduced from 37.92% to 19.32%, 13.98% to 5% and 27.20% to 12.24%, respectively. The yield of spearmint oil varies from 0.35% to 2.10% for maceration, 0.22% to 1.83% for Soxhlet and 0.07% to 0.58% for hydrodistillation with a maximum yield (2.10%) was obtained using a maceration method after 9 days of fermentation. The composition of spearmint oil has been determined and contains carvone as the major compound up to 77.88%. In addition, the diffusion coefficients for extraction of spearmint oil using the different extraction methods have been estimated and lies in the range of 2.89 x 10^{-11} m^2/s to 3.64 x 10^{-11} m^2/s. Hence, the fermentation of spearmint leaves using *A. awamori* decreased the lignocellulose content and thereby increased the yield of spearmint oil. In addition, the composition spearmint oil and diffusion coefficients of the extraction process have been determined.

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1. Introduction

Spearmint (*Mentha spicata*) is a species of mint plant (*Mentha* sp.) that contains carvone as the major compound (Znini et al., 2011). Carvone is responsible for producing a distinctive smell in spearmint oil. In 2019, the value of spearmint oil production in the United States was IDR 563 billion with a sales price of IDR 466,440 per kg of oil (USDA's, 2020). Spearmint oil is also one of the oils that is widely used in Indonesia, both in industries such as herbal medicine, soap, and toothpaste, as well as the public, making it an aromatherapy ingredient (Pribadi, 2010). However, the demand for mint oil in Indonesia needs to be supported by imports from other countries. It has been reported that the import value of mint oil to Indonesia reached up to 41.3 ton which is equivalent to IDR 14 billion (Central Bureau of Statistics, 2019). Therefore, spearmint oil production in Indonesia needs to be increased to fulfil the increasing demand of mint oil.

One of the ways to increase spearmint oil yield is optimizing the extraction process. Gavahian et al. (2015) reported an increase in the yield of peppermint oil (*Mentha piperita*) to 2.29% extracted by water

distillation as compared to steam distillation (yield of 2%). Likewise, a previous study by by Siddeeg et al. (2018) showed that the yield of peppermint oil with a maceration method (1%) and Soxhlet extraction (1.2%) was higher than the oil yield by a steam distillation method (yield of 0.9%). Another possible way to increase the yield of essential oil is through drying treatment to allow better penetration of a solvent into the cell. However, according to Rohloff et al. (2005) that has studied the drying effects of *Mentha piperita* at 30°C, 50°C and 70°C and the results showed that oil yield decreases with increasing temperature. Therefore, other treatments are needed to facilitate the extraction of spearmint oil and increase the yield. Călinescu et al. (2014) has investigated the effects of using commercial cellulase enzymes to extract essential oil from *Mentha spicata* and the results showed an increase in essential oil recovery from 0.55% to 0.58%. The cost of commercial enzymes can be reduced by using fungi that can degrade lignocellulose and increase the yield of essential oil.

One of the fungi that can be used for degradation of lignocellulose is *A. awamori*. This fungus can produce various enzymes such as cellulase, xylanase, β-glucosidase, and pectinase. These enzymes can degrade the cell walls of plants, thus facilitating the extraction process of essential oils (Gottschalk et al., 2010). According to Abduh et al. (2021), *A. awamori* was able to decrease cellulose, hemicellulose and lignin of cinnamon bark up to 16.55-39.95% after 9 days of fermentation and the yield of cinnamon oil may increase up to 200%. In another study by Janiszewska et al. (2022), *A. awamori* can be used for fermentation of olive leaves to increase phenolic compounds. Therefore, this study was conducted to determine the effect of fermentation time using *A. awamori* towards the lignocellulose content of spearmint leaves, yield and composition of the spearmint oil extracted by hydrodistillation, maceration and Soxhlet extraction methods. This study also investigated the diffusion coefficients of spearmint oil using a mathematical model.

2. Materials and methods

2.1. Chemicals and reagents

The leaves of spearmint (*Mentha spicata*) used in this study were obtained from the Al-Ittifaq Islamic Boarding School plantation located in Rancabali, Ciwidey, South Bandung, West Java – Indonesia, with two weeks old of leaves. The *A. awamori* used in this study were pure cultures obtained from the Microbiology Laboratory, Microbiology Study Program, School of Life Sciences and Technology, Institut Teknologi Bandung, West Java – Indonesia. Chemicals used in this study comprises of sodium chloride (NaCl), 80% tween, potato dextrose agar, 96% hexane, 96% ethanol, sulfuric acid (H_2SO_4), sodium hydroxide (NaOH), hydrochloric acid (HCl), and distilled water obtained from the chemical warehouse of Engineering Laboratory 1A, School of Life Sciences and Technology, Institut Teknologi Bandung, Jatinangor Campus, West Java – Indonesia.

2.2. Preparation of spearmint leaves and *A. awamori* inoculum spores

Spearmint leaves were washed and separated from the roots, stems, and impurities. Then the leaves were dried at 37-40 °C until the moisture content reaches 70% and sterilized using a UV light for 30 min (Pandey and Larroche, 2008). The preparation stage for *A. awamori* inoculum was carried out in laminar air flow under aseptic conditions. *A. awamori* fungus was inoculated on 0.78 g/ml Potato Dextrose Agar (PDA) medium for 7 days at room temperature (25-27 °C). The fungal suspension was prepared by separating the fungal spores from the PDA using an inoculating loop, then mixed in 5 ml of harvest solution consisting of 0.85% NaCl and 0.5% tween 80 (Gopalakrishnan et al., 2012).

2.3. Solid-state fermentation of spearmint leaves with *A. awamori*

The sterilized spearmint leaves were placed onto trays containing a spore solution of 0.1 ml/g of leaves with a spore concentration of 10^7 spores/ml. The mixture was stirred until a homogenous solution was attained. The fermentation was carried in a dark room (approximately 0 lux) with a moisture content of 20–70% and temperatures of 25–30 °C (Pandey and Larroche, 2008) for different incubation time (3, 6, and 9 days). The fermented spearmint leaves were sterilized with UV light for 30 min (Ikram and Dawar, 2017). Control and fermented leaves were dried inside an oven at 37-40 °C until the moisture content reached around 12% (Beigi et al., 2018). The dried leaves were cut to a size of 0.25 cm x 0.25 cm prior to the extraction process.

2.4. Extraction of essential oil from fermented spearmint leaves

After the fermentation, control, and fermented samples from different time of incubation were extracted using different methods; hydrodistillation, Soxhlet extraction and maceration. For the hydrodistillation method, the samples were placed into a distillation flask, then 600 ml of distilled water was added. The distillation was carried out in a temperature range of 85-90 °C for 4 h. As for the Soxhlet extraction method, about 10-20 g of samples were placed into the extractor, then 200 ml of hexane was added, and the extraction was carried out for 6 h followed by separation of the hexane using a rotary evaporator at 60 °C (Bimarkr et al., 2011). For a maceration method, samples were immersed in beakers containing 750 ml of 96% hexane for 24 h with agitation using a shaker followed by separation using filter papers. The filtrate was evaporated using a rotary evaporator at 60 °C to remove the hexane. The spearmint oil yield was calculated by Eq. (1).

$$Yield\ (\%) = \frac{m_{spearmint\ oil}(\mathrm{g})}{m_{dried\ leaves}\,(g)\ x\ (1-water\ content)} \mathrm{x}100\% \qquad \text{Eq. (1)}$$

For mathematical modeling, the extraction time was varied for 1, 2, 3, 4, 5 h for hydrodistillation, 3, 4, 5, 6 h for Soxhlet extraction and 3, 6, 9, 12, 18, 24 h for maceration.

2.5. Determination of lignocellulose composition

The lignocellulose composition of spearmint leaves before and after the fermentation were determined using a Chesson-Datta method as suggested by Abduh et al. (2021). A total of 1 g of spearmint (*a*) leaves was refluxed for 2 h with 150 ml H_2O at 100°C. The dried sample residue (*b*) was refluxed for 2 h with 150 ml 0.5 M H_2SO_4 at 100 °C, dried, and then calculated the sample mass (*c*). The hemicellulose content was calculated using Eq. (2). The dried sample residue was immersed in 10 ml 72% (v/v) H_2SO_4 at room temperature (25 -27 °C) for 4 h, then diluted to 0.5 M H_2SO_4 and refluxed at 100 °C for 2 h, dried, and calculated the sample mass (*d*). The cellulose was calculated using Eq. (3). The dried sample residue was heated using a furnace at temperature of 575 ± 25 °C until its mass is constant (*e*). The lignin content was calculated using Eq. (4).

$$Hemicellulose\ (\%) = \frac{b-c}{a}\ \text{x}\ 100\% \qquad \text{Eq. (2)}$$

$$Cellulose\ (\%)\ = \frac{c-d}{a}\ \text{x}\ 100\% \qquad \text{Eq. (3)}$$

$$Lignin\ (\%)\ = \frac{d-e}{a}\ x\ 100\% \qquad \text{Eq. (4)}$$

2.6. Analysis of spearmint oil

The composition of spearmint oil was determined using Gas Chromatography-Mass Spectrometry (GC-MS) at the National Police Criminal Investigation Forensic Laboratory Center, East Jakarta. Physical parameters such as color and odor were observed qualitatively by direct observation using a sensory system then compared with international spearmint oil standards (ISO 3033-1, 2005). Density measurements were carried out using Eq. (5) (Katiyar, 2017).

$$Density\ \left(\frac{g}{mL}\right) = \frac{oil\ mass\ (g)}{oil\ volume\ (mL)} \qquad \text{Eq. (5)}$$

2.7. Determination of diffusion coefficient

The relationship between spearmint oil yield with extraction time can be estimated using Fick's second law as shown in Eq. (6) as suggested by Abduh et al. (2021).

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial y^2} \qquad \text{Eq. (6)}$$

C (g/m^3) is the mean concentration of essential oil in the samples at time t (s), y (m) is the displacement along the diffusion direction and D (m^2/s) is the diffusion coefficient. Eq. (6) was solved and simplified to Eq. (7).

$$\frac{M_t\ (\%)}{M_\infty\ (\%)} = 1 - Ae^{(-B(s^{-1})\ x\ t(s))} \qquad \text{Eq. (7)}$$

M_t is the yield at time t, M_∞ is the yield at steady state, A is the model constant (A = 8/π^2 for plate geometry), t is time, and B is the diffusion rate constant. Eq. (7) can be transformed to Eq. (8) by changing the yield fraction of cinnamon oil to a constant E.

$$E = 1 - \frac{M_t\ (\%)}{M_\infty\ (\%)}\ = Ae^{\left(-B(s^{-1})\ x\ t(s)\right)}$$

$$\ln \text{E} = \ln \text{A-B}(\text{s}^{-1})\ \text{x t (s)} \qquad \text{Eq. (8)}$$

Eq. (8) was solved using MATLAB to estimate the B parameter using the curve fitting. The k parameter was used to determine the diffusion coefficient (D) using Eq. (9) where L is the width of the particles.

$$\text{D}\ \left(\frac{\text{cm}^2}{\text{s}}\right) = \frac{\text{B}(\text{s}^{-1})\ \text{x L(cm)}^2}{\pi^2} \qquad \text{Eq. (9)}$$

3. Results and discussion

3.1. Effects of lignocellulose biodegradation by *A. awamori* on lignocellulose content of spearmint leave and spearmint oil yield.

In this study, a solid-state fermentation system was used with *A. awamori* as a biological agent that can produce lignocellulose degrading enzymes such as cellulase, xylanase, endo-polygalacturonase (endo-PG), exo-polygalacturonase (exo-PG), and *laccase* (Haranath et al., 2017). Spearmint leaves used in the fermentation process had a water content of 60-70%. As such is based on the results by Padma et al. (2012) that the highest enzyme activity produced by *A. awamori* in a solid-state fermentation system was obtained at a water content of 65%. In another study, Pandey (2008) also observed that water content that supported fungal growth lies in the range of 20-70% (Pandey, 2008).

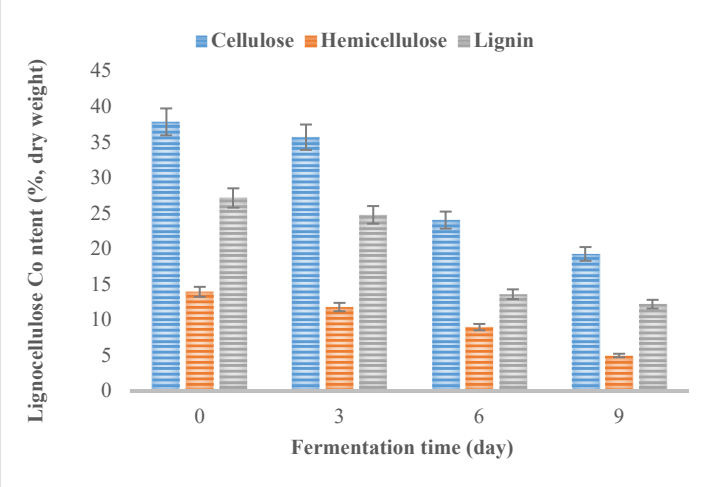

Fig. 1. Effects of fernentation using using *A. awamori* on lignocellulose content of spearmint leaves

Cellulose content in the control samples (day 0) was 37.92 ± 4.16% dry weight. After the fermentation using *A. awamori*, the cellulose content decreased to 35.76 ± 4.77% (3rd day), 24.08 ± 2.51% (6th day), and 19.32 ± 1.02% (9th day) dry weight as shown in Fig 1.. The decrease occurred due to the biodegradation process of cellulose by *A. awamori* which could produce endoglucanase, β-glucosidase, and exoglucanase enzymes. The three hydrolysis enzymes synergize with each other to degrade cellulose (Ikubar et al., 2018). The yield of spearmint oil was inversely related to cellulose content. This finding is consistent with the previous study by Călinescu et al. (2014) that enzymatic pre-treatment using cellulase enzymes on spearmint leaves could increase the yield of spearmint oil from 0.55% to 0.58% dry weight.

Hemicellulose content in the control samples (day 0) was 13.98 ± 1.53% dry weight. After the fermentation process using *A. awamori*, the hemicellulose content obtained was 11.83 ± 1.25% dry weight on the 3rd day of fermentation, 9.00 ± 2.83% dry weight on the 6th day of fermentation, and 5.9 ± 1.52% dry weight on the 9th day of fermentation. The decrease in hemicellulose content is due to the degradation of xylan with the help of the enzyme xylanase secreted by *A. awamori* (Ikubar et al., 2018).

Lignin content in the control samples (day 0) was 27.20 ± 0.10% dry weight. After the fermentation process using *A. awamori*, the cellulose content decreased to 24.82 ± 0.85% dry weight on the 3rd day, 13.64 ± 1.52% dry weight on the 6th day, and 12.24 ± 0.47% dry weight on the 9th day. The degradation process caused the decrease of lignin levels by lignin peroxidase, manganese peroxidase, and laccase which secreted by *A. awamori*. The significant reduction on day 6 is consistent with the study results by Haranath et al., (2017) that the highest laccase activity produced by *A. awamori* using the substrate fermentation system was 32 IU/ml occurred on the sixth day. Based on the results obtained, the spearmint oil yield is inversely related to lignin levels.

In this study, the yield of spearmint oil using maceration, Soxhlet, and hydrodistillation methods were determined and compared to each other. Based on Fig 2., the yield of spearmint oil using the maceration method tends to be higher than the others, with the highest gain being 2.10 ± 1.09% dry weight on the 9th days of fermentation. The difference in operating temperature and the solvent were thoughts to affect that result. The maceration used a relatively low temperature compared to Soxhlet and hydrodistillation, so it would minimize the possibility of losing volatile compounds.

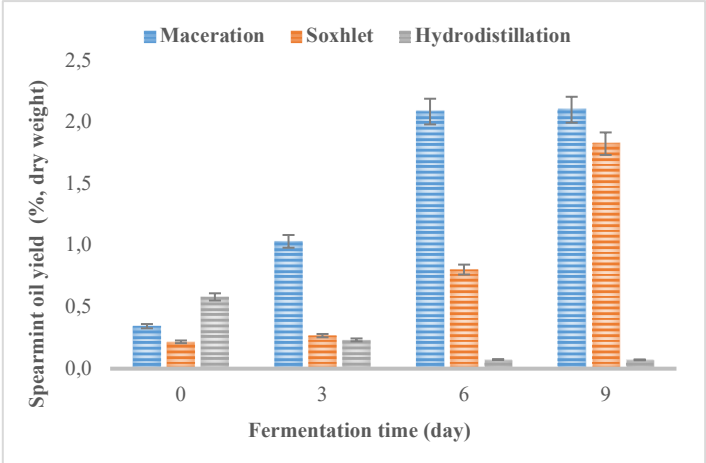

Fig. 2. Effects of fermentation using *A. awamori* on spearmint oil yield extracted from spearmint leaves using different extraction methods.

Table 1. Characteristics of spearmint oil

Characteristics	Isolation method			ISO 3033-1:2005
	Maceration	Soxhlet	Hydrodistillation	
Color	Yellowish clear	Yellowish clear	Yellow	Almost colorless to pale yellow
Odor	Herbaceous note with the smell of fermentation	Herbaceous note with the smell of fermentation	Herbaceous note with the smell of fermentation	Characteristics odor of carvone with an herbaceous note
Density (g/ml)	1.07 ± 0.02	0.93 ± 0.003	0.93 ± 0.06	0.92 - 0.94

The yield of spearmint oil using maceration and Soxhlet methods showed a similar trend. The hydrodistillation method showed the opposite result where the yield trend tends to decrease with the length of fermentation time. That result could occur because of the volatile compounds that evaporated during the drying and fermentation process (Sugiarto and Hamidi, 2019). Moreover, the temperature used in the extraction process tends to be very high, reaching 90 °C.

3.2. Characteristics of spearmint oil

Physical characteristics in the form of color, aroma and density of the obtained spearmint oil were compared with the international standards established by the International Organization for Standardization (ISO) as shown in Table 1. The color characteristics are in accordance with those specified by ISO, while the aroma that smells varies considerably with each treatment. For the control samples (day 0) and fermented samples for 3 days, the odor resembled the description of odor characteristics specified by ISO. On the 6th and 9th days of the fermentation treatment, the distinctive odor of the carvone was not very much smelled, then the odor of the remains of fermentation was still smelled.

The density of spearmint oil obtained from the Soxhlet and hydrodistillation method are in agreement with the specified International Organization for Standardization (ISO). Density is intensive property, which means that increasing the amount of substance will not increase the density value (Canagarata and Sebastian, 1992). One of the factors that affect density is molecular geometry. Based on the results of the GC-MS analysis as shown in Table 2, it is known that the spearmint oil components obtained were different in each treatment and there was no major compound (carvone) found in some samples. Overall, spearmint oils contains various types of compounds such as monoterpenes, sesquiterpenes, terpenes, triterpenes, phenols, steroids and fatty acids. The major compounds in spearmint leaves are monoterpenes such as carvone, limonene, carveol, and 1,8-cineol (Snoussi et al., 2015). The composition of spearmint oil obtained in this study is slightly different from the reported values in in the literature. This could be due to differences in the location of spearmint cultivation, which have different geographic and meteorological conditions (Telci et al., 2010). The spearmint used in the research by Snoussi et al., (2015) was cultivated in Tunisia, which has a Mediterranean climate. The age of the plant, the moisture content of the leaves and the method of drying are known to be other factors that can affect the content of spearmint oil compounds (Smigielski et al., 2011; Zheljazkov et al., 2010).

Table 2. Composition of spearmint oil as determined by GC-MS

Compounds	The number of compounds (%)												
	Control			3rd day fermentation			6th day fermentation			9th day fermentation			Ref*
	M[1]	S[2]	HD[3]	M[1]	S[2]	HD[3]	M[1]	S[2]	HD[3]	M[1]	S[2]	HD[3]	
D-carvone	75.01	2.84	77.88	18.4	nd	74.64	nd	3.13	6.22	10.29	nd	nd	29-59
cis-carveol	3.08	4,24	0.42	nd	nd	1,60	nd	nd	1.41	nd	nd	1.16	0.60
cis-dihydro carvone	nd	nd	1.29	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.50
neo-iso-dihydrocarveol	nd	nd	nd	1.24	nd	5.92	nd	nd	1.92	nd	nd	1.95	0.22
Dihydrocarveol	1.77	nd	nd	4.67	nd	nd	nd	nd	nd	nd	nd	nd	1.70
β-bourbonene	nd	nd	1.44	nd	nd	1.84	nd	31.2	2.33	nd	nd	nd	2.90
1,8-cineole	nd	nd	nd	0.77	21,6	nd	nd	nd	nd	nd	nd	nd	17.0
Β-copaene	nd	nd	nd	nd	nd	0.27	nd	nd	0.46	nd	nd	1.29	nd
o-Cymene	nd	2.23	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	6.00
Germacrene D	nd	nd	nd	nd	nd	0.28	nd	nd	3.54	nd	nd	17.69	3.90
Caryophyllene	nd	nd	nd	nd	nd	1.21	nd	nd	2.76	nd	nd	8.24	3.20
Caryophyllene oxide	nd	nd	1.29	nd	nd	1.33	nd	nd	0.51	nd	nd	nd	0.90
Limonene-1,2-diol	nd	nd	nd	3.79	nd	nd	nd	nd	nd	nd	nd	nd	nd
Piperitenone	1.27	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.15
α-Cadinol	nd	nd	0.65	3.17	nd	nd	nd	nd	1.06	nd	nd	1.81	0.47
trans-calamenene	nd	nd	0.44	nd	nd	0.86	nd	nd	2.11	nd	nd	4.46	nd
Jasmone	nd	nd	nd	1.69	nd	nd	nd	nd	nd	nd	nd	nd	0.63
epi-cubenol	nd	nd	0.52	nd	nd	0.98	nd	nd	1.06	nd	nd	2.03	0.20
Farnesene (cis-beta)	nd	nd	nd	nd	nd	0.57	nd	nd	0.46	nd	nd	2.06	0.60
α-ionone epoxide	nd	3.95	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	14.0
trans-isopulegone	nd	2.79	nd	1,68	nd	nd	nd	nd	nd	nd	nd	nd	nd
Dihydroactinolide	1.45	nd	nd	1,12	nd	nd	nd	nd	nd	1.48	2.82	nd	nd
Hexylene glycol	0.02	nd	nd	0,63	nd	nd	nd	nd	nd	2.61	2.90	nd	nd
Hexadecanoic acid	nd	nd	0.48	nd	nd	0.30	1,27	nd	0.94	11.04	nd	nd	nd
Teresantalol	nd	nd	nd	4.76	nd	nd	nd	22.8	nd	nd	43.8	nd	nd
4-Cyclohexylidenebutyraldehyde	nd	12.9	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Methyl-d3-thioanisole	nd	5.63	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Diethylene glycol	nd	4.82	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
4'-Fluoro-2'-nitroacetanilide	nd	7.78	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
cis-p-mentha-1(7),8-dien-2-ol	nd	4.08	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Diethylene glycol monobutyl ether	0.98	nd	nd	4.80	nd	nd	nd	nd	nd	nd	13.4	nd	nd
19-Dimethano-10,14-metheno-26,30-nitrilo 25Hdibenzo	nd	nd	9.46	nd	nd	2,48	50,69	nd	37.3	0.27	nd	16.7	nd
Octadecanamide	nd	nd	nd	nd	23.9	nd	nd	nd	nd	17.04	nd	nd	nd
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	0.20	nd	nd	1.72	nd	nd	nd	nd	nd	8.24	nd	nd	nd
Cyclopropanemethanol, 2-isopropylidene-.alpha.-methyl-	nd	nd	nd	4.30	nd	nd	nd	nd	nd	8.65	nd	nd	nd
2,4-Di-tert-butylphenol	nd	nd	nd	5.32	nd	nd	nd	nd	nd	nd	nd	2.39	nd
Other compounds	16.22	48.8	6.13	41.9	54.4	5.72	48.04	42.9	37.9	40.38	37.1	40.22	

[1]Maceration; [2]Soxhlet; [3]Hydrodistillation; nd: not detected

*Zheljazkov et al. (2010); Znini et al. (2011); Snoussi et al. (2015); Cirlini et al. (2016)

The composition of spearmint oil with the Soxhlet method has differences in the number of compounds compared to maceration, hydrodistillation, and literature. Possibly, this may be due to less volume of solvent used for Soxhlet extraction (200 ml) as compared to maceration (750 ml) and hydrodistillation (600 ml) and which limits the mass transfer of carvone. The main compound of spearmint oil, carvone, decreases with the length of fermentation time. The highest carvone compound was obtained from the oil during the fermentation on the 0th day using hydrodistillation method (77.88%). The carvone content was higher compared to the research of Znini et al., (2011) which was 29%. The percentage of carvone obtained using maceration for each treatment was 75.01% for the control samples (day 0), 18.36% for fermentation on day 3 and 10.29% for fermentation on day 9. During the fermentation treatment for 6 days, no carvone was detected.

The decrease in carvone content can occur due to evaporation when the leaves are left in the open-air during fermentation. The evaporation rate of carvone tends to be higher than other components of spearmint oil in the open air (Salim et al., 2015). This is supported by the research results of Salim et al., (2015) which showed that spearmint leaves left in the open air for seven days contained the lowest carvone content (69.65%) compared to samples

left for three days (73,34%) and without being left in the open air (82.06%). Other causes of loss of constituents in spearmint oil include oxidation or resinification reactions (Guenther, 1975). In spearmint oil samples extracted using maceration, the resin was found in the storage bottles of 6th and 9th fermentation days caused by the resinification reaction, so it was thought to affect the results of the GC-MS analysis. Nevertheless, further studies are required to really understand the reasoning for the decreasing amount of carvone in the extracted spearmint oil throughout the fermentation process. Additional studies are also interested to be carried out to determine the optimum fermentation time for greater yield and quality of spearmint oil.

3.3. Estimation of diffusion coefficient

The mass transfer process of oil from the spearmint leaves during the extraction process occurs by diffusion. In the extraction process, the diffusion process is unstable, the concentration of oil particles will change over time and distance. Therefore, the diffusion process can be described mathematically by Fick's second law and the results are shown in Table 3. The value of the *k* parameter then used to determine the value of the diffusion coefficient using Eq. (8). The table shows that every *D* value of each method is not much different. Likewise, the *D* value obtained from the process of extracting *Mentha arvensis* leaves using the steam distillation method by Katiyar (2017) was 1.027 x 10^{-11} m^2/s. The difference in diffusion coefficient can occur due to different plant varieties, extraction conditions such as temperature, stirring, and extraction method (Petrovic et al., 2012).

Table 3. Diffusion coefficients for isolation of spearmint oil

Method	k (s^{-1})	D (m^2/s)
Hydrodistilation	4.56 x 10^{-5}	2.89 x 10^{-11}
Maceration	4.87 x 10^{-5}	3.09 x 10^{-11}
Soxhlet	5.74 x 10^{-5}	3.64 x 10^{-11}

4. Conclusion

The pre-treatment of fermentation using *A. awamori* could increase spearmint oil yield. The yield of spearmint oil using maceration and Soxhlet tends to increase against the length of fermentation time which correlated to the decrease of lignocellulose content. The highest spearmint oil yield was 2.10% dry weight which occurred during the 9th days of fermentation using the maceration method. The highest decrease in cellulose and lignin levels occurred during fermentation for 9th days, while hemicellulose at 6th days with a decrease of 49.05%, 62.65%, and 34.40% respectively to the control. The length of fermentation time can affect the composition of spearmint oil. Each fermentation time and extraction method variations have different spearmint oil components. Carvone is the main compound in spearmint oil. The percentage of carvone tends to decrease with the length of fermentation time. Carvone content of spearmint oil with fermentation treatment for 0-9 days lies in the range of 2.84 - 77.88%. The estimated diffusion coefficient obtained for hydrodistillation, maceration, and Soxhlet method were 2.89 x 10^{-11} m^2/s, 3.09 x 10^{-11} m^2/s, and 3.64 x 10^{-11} m^2/s, respectively.

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Conflict of interest

The authors declare there is no conflict of interest in this study.

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