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Effect of microwave pretreatment method on essential oil extraction on key lime peel (Citrus × aurantiifolia)

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Abstract: *The peel of key lime is regarded as a byproduct that contains a substantial amount of bioactive components, including essential oil. The extraction of essential oils from key lime peel typically involves conventional techniques such as hydro-distillation or solvent extraction, which are known for their time-intensive and energy-intensive. The employing of microwave pretreatment has emerged as a conventional approach in the extraction of essential oils from various plant materials. The objective of this study was to investigate the optimization of the effect of microwave pretreatment method on essential oil of key lime peel. Response surface methodology based on Face-Centered CCD was applied to optimize the parameters for microwave pretreatment such as microwave power (600 – 800 W), irradiation time (2 – 5 min) and solid-to-solvent ratio (SSR) (1:0 – 1:2 w/v) . The essential oil extracts at optimized microwave pretreatment conditions were compared with hydro-distillation. The optimized condition of microwave pretreatment was 1000 W microwave power, 2 min irradiation time and SSR of 1:0 w/v. The analysis of ANOVA and three-dimensional response surface revealed the primary factor of microwave pretreatment conditions that affects the yield of essential oil was the SSR, follow up by irradiation time and microwave power. The essential oil was subjected to analysis using GC-MS, revealing the presence of 39 compounds in both the essential oils obtained from pretreatment and non-pretreatment using microwave. No discernible disparities were observed in the composition of essential oil under all of those conditions.*

Keywords: Microwave, Pretreatment, Extraction, Essential oil, Lime peel

1. INTRODUCTION

Key lime (*Citrus* × *aurantiifolia*) is widely consumed and cultivated around the world for their refreshing taste and nutritional benefits. The worldwide production of lemon and limes reached a total of around 20,529,600 tons and approximately 6% of lemons and limes were utilized for juice processing in 2019 [1]. Following the processing phase, the citrus industries generate substantial quantities of waste, as the citrus peel waste constitutes roughly 44% of the total fruit mass [2]. However, it constitutes a significant reservoir of bioactive constituents, such as essential oils, flavonoids, carotenoids, and phenolic acids [3]. Those bioactive compounds have been traditionally used in various industries, including food, cosmetics, and pharmaceuticals [4], as their exhibits properties, including antioxidant, anti-inflammatory, anticancer, and antibacterial activities [5].

The process of extracting essential oils from key lime peel typically involves conventional techniques such as hydro-distillation or solvent extraction. These methods are considered to be time-intensive and energy-intensive [6]. Microwave pretreatment is a relatively new method for the extraction of essential oils from plant materials. It involves subjecting the plant material to microwave radiation before the conventional extraction process. Microwave pretreatment works by rupturing the cell walls of the plant material and facilitating the release of the essential oil [7]. The method has gained popularity and is considered to be more efficient than traditional extraction methods, as it requires less energy, decrease the time required for essential oil extraction and

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increase the yield of essential oils [8]. Despite its benefits, microwave pretreatment also has some drawbacks. One of the main limitations of the technique is the potential for overheating and degradation of the essential oil. The use of high temperatures during microwave pretreatment can cause chemical compounds change in the essential oil, which can affect its quality and aroma [9]. Therefore, it is important to carefully control the temperature and duration of the microwave pretreatment to avoid damaging the essential oil. The thermal impact of microwave technology is influenced by a range of variables, which can be categorized into two groups: microwave-related factors and material-related factors. The variables that affect microwaves are comprised of microwave power, exposure duration, radio frequency, and power density. The material factors encompass various aspects such as dielectric properties, moisture, penetration depth, and geometry. The microwave heating effect is influenced by a multitude of interacting factors [10]. Due to the numerous variables that affect microwave pretreatment, it is necessary to optimize the process parameters in order to preserve the maximum extraction of essential oils. Response surface methodology (RSM) is a statistical tool that is particularly appropriate for optimize multifaceted processes and determine the effects of multiple variables and their interactions so that the response reaches a desired maximum or minimum value [11].

This research study aims to optimize the effect of microwave pretreatment on key lime peel for the purpose to increase the yield of essential oil extraction. Response surface methodology based on Face-Centered Central Composite Design (FCCD) was applied to investigate and optimize the pretreatment process parameters such as microwave power, irradiation time, and solid-to-solvent ratio (SSR) on the maximum yield of essential oil. The chemical composition of the essential oil was analyzed by gas chromatography-mass spectrometry (GC-MS). Furthermore, the results were compared with non-microwave pretreatment essential oil extraction.

2. Methodology

2.1 Sample collection and preparation

Fresh key limes used in this study were purchased from Pursat province of Cambodia. The key limes were rinsed and sanitized to remove dust and foreign objects, then peeled. The size of key lime peel was approximately 1 cm^2, and then the peel was stored in Ziplock bag at -20°C until being used in future processing.

2.2 Experimental design

The effect of microwave pretreatment conditions on the yield of essential oil (EO) was determined by applying a response surface methodology, using a Face-Centered central composite design (FCCD). To determine the optimum conditions, three independent variables were considered such as X_1: microwave power (600, 800, and 1000 W), X_2: irradiation time (2, 3.5, and 5 min), and X_3: SSR (w/v) (1:0, 1:1, and 1:2) see **in Table 1.**

Table 1. FCCD experiment design condition for the effect of microwave pretreatment of key lime peel essential oil extraction

Independent variables	X_1: Microwave power (W)	X_2: Irradiation time (min)	X_3: Solid-to-solvent ratio (w/v)
Low	600	2	1:0
Medium	800	3.5	1:1
High	1000	5	1:2

Through the elaborates the arrangement of the FCCD in this research, a total of 16 different combinations including: 8 factorial experiments, designated by the coded variables (-) or (+), 6 axial experiments, defined by the coded variables (a) or (A), 2 central experiments, specified by the coded variable 0, which were employed to fit the full second-order polynomial equation mode. The second order polynomial, generally used for the composite designs, is given in Eq.1.

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i<j}^{n} \beta_{ij} X_i X_j + \sum_{i=1}^{n} \beta_i X_i^2 + \varepsilon \qquad \text{(Eq.1)}$$

Where Y is the predicted essential oil yield, β_0 is a constant, β_i , β_{ii} and β_{ij} are the regression coefficients for linearity, quadratic and interactive terms respectively, while X_i and X_j are levels of the independent variables and ε is the residual associated to the experimental data [12].

2.3 Procedure of microwave pretreatment and essential oil extraction

The microwave pretreatment was done by microwave model NN-GD37HB Panasonic. About 100 g sample was placed in a microwaveable container, added ratio of distilled water, set microwave power and time followed the experimental design (see **Table 2** and **Fig. 1**) and then microwaved. The SSR levels were range from 1:0, 1:1, and 1:2 (w/v), the power levels of microwave were 600, 800 and 1000 W, and microwave heating times were 2, 3.5, and 5 min.

After the pretreatment, the sample was allowed to cool down, then grinded and distilled water was added up to 400 mL, before starting essential oil (EO) extraction process by hydro-distillation extraction using Clevenger-type apparatus. The non-pretreatment, 100g of sample were grinded and mixed with 400 mL of distilled water before placed directly to hydro-distillation for EO extraction. The obtained EO was weighted and recorded the yield.

$$Essential\ oil\ yield\ (\%w/w) = \frac{Mass\ of\ essential\ oils}{Mass\ of\ sample} \times 100 \quad \text{(Eq.2)}$$

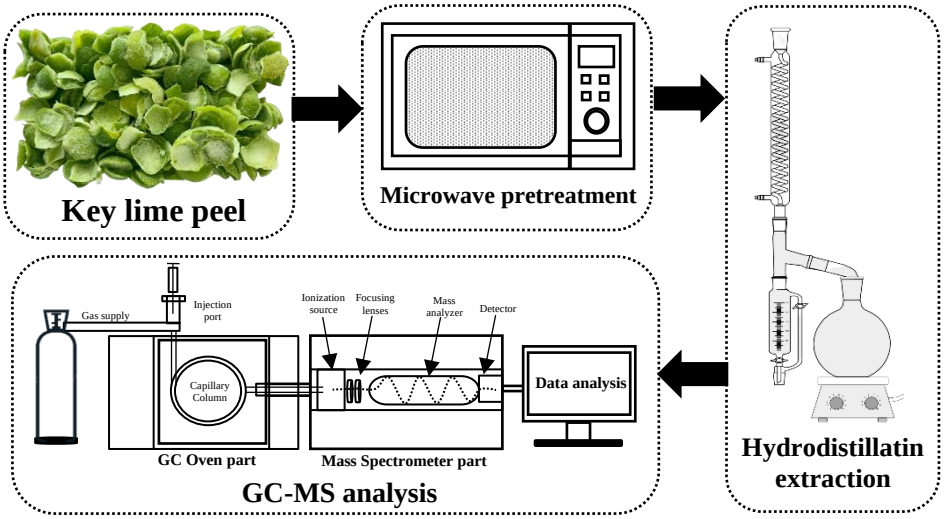


Fig. 1 Diagram of experimental study

2.4 Volatile compound analysis

The gas chromatography–mass spectrometry (GC–MS) model was used to analyze the essential oil (EO) in the peel of *Citrus aurantiifolia*. A TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA) and a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer) were used to construct the equipment. The Thermo Scientific TM TG-5MS GC Columns (0.25mm x 60m, 0.25 m film thickness) were used in the GC–MS device. EO samples were diluted by mixing 10 mg with 1 ml of n-hexane. Analyses were conducted with helium as the carrier gas at a flow rate of 1.0 mL/min and a split ratio of 1:10, using the following temperature program 60 °C for 1 min, rising at 3 °C/min to 240 °C, and holding for 1 min. The injector and detector were held at 240 °C. Diluted samples of 0.2 µL were always inject. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40–450. The identification of individual compounds was achieved through a comparative analysis of their mass spectral data against the NIST 11 library database, coupled with the provision of corresponding retention times. The peak area or area normalization was determined through the comparison of a particular compound with the overall identified compounds [13].

3. RESULTS AND DISCUSSIONS

3.1 Yield of essential oil

The application of microwaves as a pretreatment technique aimed to enhance the essential oil (EO) yield. As the result, the yield of EO was obtained by using microwave pretreatment, it had increased about 19.32 ± 0.01% compared to the yield of EO of non-pretreatment key lime peel (from 1.3976 ± 0.003 to 1.6676 ± 0.017%). This result is similar to a study of the extraction process of essential oil from orange peel using microwave assisted extraction shown that the EO yield were achieving within the range of 0.9% to 2.5%, which were higher than the EO obtained by hydro-distillation extraction system [14].

Table 2. shows the EO yields of key lime peel affected by microwave pretreatment conditions at various levels of microwave power (600, 800, and 1000 W), microwave heating time (2, 3.5, and 5 min), and SSR (1:0, 1:1, and 1:2 w/v). Through the microwave pretreatment method, it is found that EO yield were ranged from 1.34% to 1.96% (w/w). The highest yield of EO was found at pretreatment condition at microwave power of 1000 W, microwave heating time of 2 min and SSR level of 1:0 w/v. Conversely the lowest EO yield was obtained at microwave power of 600 W, irradiation time of 2 min and SSR of 1:2 w/v. The results of this study are probably subject to the impact of various pretreatment conditions, including microwave power, irradiation time, and SSR, as well as the potential interaction effect of these specific pretreatment parameters [15].

Table *2.* Microwave pretreatment conditions for all the 16 runs performed according to the Face-Centered CCD (FCCD)

Run	Microwave power (W)	Irradiation time (min)	Solid-to-solvent ratio (w/v)	EO yield (% w/w)
1	600	2	1:0	1.909
2	600	2	1:2	1.3446
3	600	5	1:0	1.5664
4	600	5	1:2	1.7963
5	1000	2	1:0	1.9596
6	1000	2	1:2	1.5542
7	1000	5	1:0	1.3496
8	1000	5	1:2	1.602
9	800	3.5	1:1	1.6852
10	800	3.5	1:1	1.7097
11	600	3.5	1:1	1.7987
12	800	2	1:1	1.6089

13	800	3.5	1:0	1.6452
14	800	3.5	1:2	1.6565
15	800	5	1:1	1.6049
16	1000	3.5	1:1	1.8909

3.2 Linear effect of variables

Fig 2. shows the linear regression between yield of essential oil (EO) to parameter of microwave pretreatment conditions as: **A** describes the effect of microwave power on the yield of EO (600, 800, and 1000w), **B** describes the effect of irradiation time on the yield of EO (2, 3.5, and 5min), **C** describes the effect of SSR on the yield of EO (1:0, 1:1, and 1:2 w/v). Overall, the irradiation time and SSR (w/v) shared a similar trend of the line graph as EO yield rose moderately from 1.67 to 1.73% as irradiation time increased from 2 to 3.5 min, as well as SSR increased from 1:0 to 1:1, EO yield had increase from 1.68 to 1.72%. The declined of EO yield was observed from 3.5 to 5 min, as EO yield decrease from 1.73 to 1.58% and SSR from 1:1 to 1:2, as EO yield decrease from 1.72 to 1.59%. A similarly results also obtain from a study of Microwave-Assisted Extraction of EO from Vietnamese Basil [16], which shown that the irrational times from 30 to 90 min and SSR from 1:1 to 1:3 (g/mL) resulted in higher yields of EO from 0.2 to 0.7%, while the prolonged time more than 90 and higher level of SSR more than 1:3, leaded to decrease of EO yield from 0.7 to 0.4%. The longer exposed times can lead to higher yields of EO, as it allows more time for the EO components to diffuse out of the plant material and into the extraction solvent. However, there is a limit to how much EO can be extracted, and excessively long extraction times can lead to the extraction of unwanted compounds and degradation of EO components. As well as for SSR, lower SSR improve the contact surface between plant material and solvent, thereby promoting the mass transfer of soluble chemicals from material to solvent. Nevertheless, the excessively higher of the SSR can be corresponded in the excessive extraction solvent, take a long time to concentrate, more dissolved out impurities such as polysaccharide and slowing the solubility of target compounds [17].

Conversely, the graph of microwave power shows no significant effect on EO yield since it increased from 1.65 to 1.67% ($p > 0.05$) within the microwave power of 600 to 1000w. In broad terms, power of a microwave oven refers to the amount of electrical power that the microwave's magnetron is able to generate and deliver to the sample. The power of a microwave oven affects how quickly it is able to cause the water molecules to vibrate rapidly, which generates heat in the sample [18]. Subsequently, thermal energy affects the structure and composition of the plant material by soften or rupture plant cells, making it more or less amenable to release the EO [19]. In this case, the microwave power shown insignificant effect on the EO yield ($p < 0.05$). The likely reason for the limited impact on the yield of key lime peel essential oil in the experiment is attributed to the insufficient power level employed, and the structure of the key lime peel and the chemical composition of its essential oil make it less susceptible to changes in temperature and pressure caused by microwave irradiation compared to other plant materials [20].

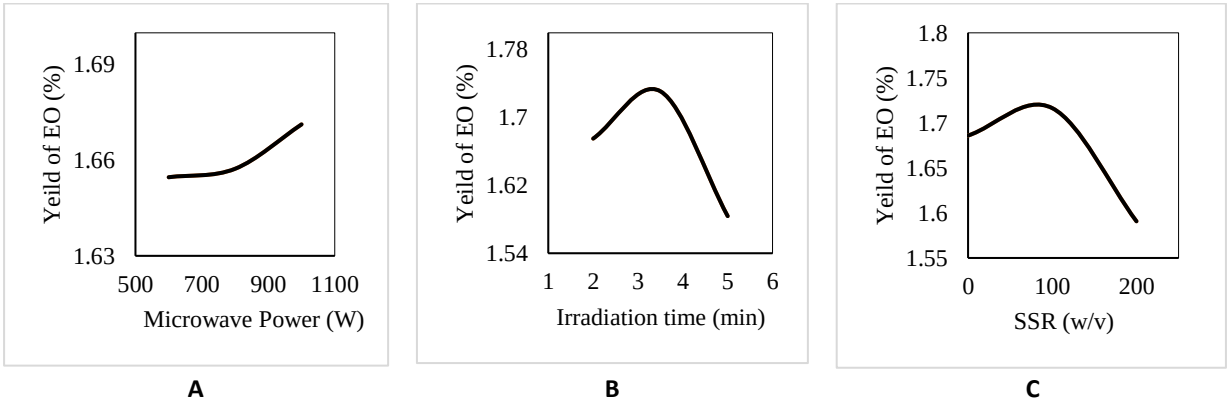


Fig. 2. Linear graphs of the effect of microwave pretreatment on essential oil yield of key lime. (A) the effect of microwave power, (B) the effect of irradiation time, and (C) the effect of SSR on the yield of essential oil.

3.3 Model fitting and statistical analysis

The observed data was analyzed by analysis of variance (ANOVA) and the results were shown in **Table 3.2**. The significance of the developed model equations was evaluated by their corresponding *p*-values and *F*-value. The lower *p*-value ($p < 0.005$) of the model demonstrated that the developed model was very convincing significance. The *F*-value of the model was estimated to be 13.14 specifying that the model was significant. In this model, the *F*-value for lack-of-fit was estimated to be 0.1939 ($p > 0.005$) implying that the lack-of-fit was insignificant. The determination coefficient (R^2) meant the proportion of the total variation in the response expected by the model. In this study, the determination

coefficient (R^2) was 0.9517 suggesting a 95.17% match between the predicted and experimental data. Meanwhile, the value of adjusted determination coefficient (Adj R^2 =0.8792) was also very high, indicating a high significance of the model developed through experimental data. The smaller the coefficient of variance (C.V.%) was, the more reliable the model would get. The coefficient of variance value of 3.66% showed that the deviations between experimental and predicted values are low and also showed a high degree of precision and reliability of the conducted experiments. It was found that one linear term of SSR (X_3, $p < 0.05$), two interaction terms between microwave power with time (X_1X_2, $p < 0.05$) and microwave heating time with SSR (X_2X_3, $p < 0.05$), as well as, two quadratic terms of them (X_1^2, $p < 0.05$), and (X_2^2, $p < 0.05$) had a significant effect on the yield of essential oil. While linear effect of microwave power (X_1, $p > 0.05$), interaction effect of microwave power with SSR (X_1X_3, $p > 0.05$), and quatatic effect of SSR (X_3^2, $p > 0.05$) were not significant. The model of extraction yield value could be expressed by the following second order polynomial equations equation (3.1):

$$y = 1.717 - 0.006X_1 - 0.046X_2 - 0.048X_3 - 0.084X_1X_2 + 0.023X_1X_3 + 0.182X_2X_3 + 0.117X_1^2 - 0.121X_2^2 - 0.077X_3^2 \quad \text{(Eq. 3.1)}$$

In table 3. some variables were insignificant and were removed to make up the new model.

$$y = 1.717 - 0.048X_3 - 0.084X_1X_2 + 0.182X_2X_3 + 0.117X_1^2 - 0.121X_2^2 \quad \text{(Eq. 3.2)}$$

Where Y is the essential oil extraction yield, X_1 is the microwave power, X_2 is the irradiation time, X_3 is the water-to-solid ratio. Experimental data were analysed using JMP software (Version 16.0.0) and fitted to a second-order polynomial regression model containing the coefficient of linear, quadratic, and three factors interaction effects. ANOVA was used to analyze the model for significance and suitability.

Considering the feasibility of operation, the experimental conditions was modified to microwave power of 1000 W, irradiation time of 2 min and absent of added water. The maximum predicted yield of essential oil was 1.97%. Verification experiments were performed two times under the optimized conditions and the mean extraction yield was 1.96% with a relative standard deviation (RSD) of 0.0071%. The predicted values were very close to the actual values, indicating the models established were reasonable and reliable.

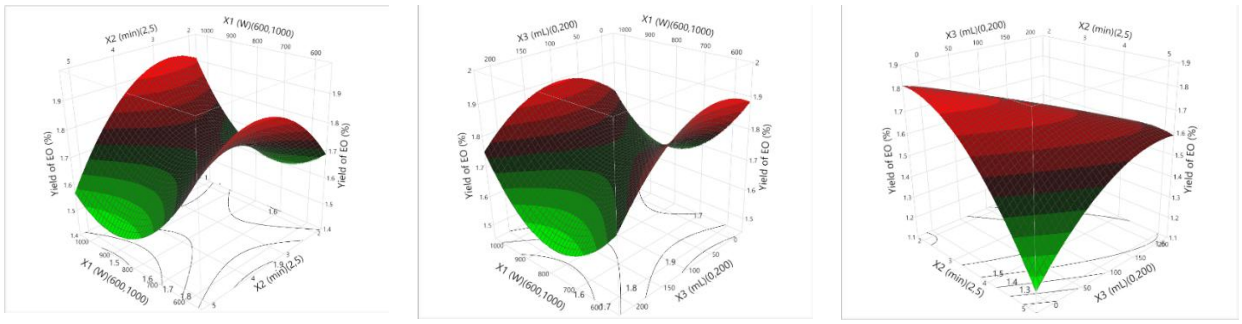
Table 3. Analysis of variance (ANOVA) for response surface quadratic model.

Sources	Sum of square	Degree of Freedom	F-value	*p*-value
Model	0.447	9	13.14	0.0027
Lack of fit	0.022	5	14.927	0.1939
X_1	0.0003	1	0.091	0.773
X_2	0.021	1	5.523	0.0571
X_3	0.023	1	5.994	0.0499*
X_1X_2	0.056	1	14.889	0.0084**
X_1X_3	0.004	1	1.088	0.337
X_2X_3	0.263	1	69.669	0.0002**
X_1^2	0.036	1	9.601	0.0212*
X_2^2	0.038	1	10.123	0.019*
X_3^2	0.015	1	4.086	0.0897
R^2 = 0.9564 Adjust R^2 = 0.8868 C.V.% = 3.66%				

Note: (1) represents microwave power, (2) is irradiation time, and (3) is volume of added water. * is significant, ** is very significant, and *** is highly significant.

3.4 Responses surface and graph analysis

A three-dimensional response surface (as shown in **Fig 3**) was generated specifically to evaluate the linear and quadratic effects, as well as the interaction effects of the independent variables such as microwave power (X1), irradiation time (X2), and solid-to-solvent ratio (X3) on the yields of essential oils. The coloration of a dark red color is indicative of the highest yield of essential oil (EO), while green color is indicative of the lowest yield. **Fig 3.** demonstrate the 3D of response surface plots of the EO yield from key lime by using microwave pretreatment. Overall, **Fig 3A** and **B** shared a similar trend of the three-dimensional response surface graph.



A B C

Fig. 3. Surface plots of the yield essential oil from key lime by using microwave pretreatment. (**A**) interaction effects of the microwave power and irradiation time on the yield of essential oil, (**B**) interaction effects of the microwave power and solid to solvent radio on the yield of essential oil, (**C**) interaction effects of the irradiation time and solid to solvent radio on the yield of essential oil.

Fig 3A describes the interactive effect of microwave power and irradiation time at fixed SSR (1:1w/v). The graph indicates the microwave power factor was dependent on the time variable ($p < 0.05$). When the power of the microwave was increased from 600 to 1000W, and the time decreased from 5 to 2 minutes, there was a statistically significant increase in the yield of EO. In contrast, it has been observed that an increase in both microwave heating time and microwave power leads to a major reduction in the yield of EO. This phenomenon has a similarity to research of the extraction of EO influenced by the interaction between microwave energy and irradiation time. The EO yields have been observed to improve with an increase in microwave power over a shorter duration. However, it has been observed that a further increase in microwave power over a prolonged duration leads to a decrease in EO yields [21]. It is crucial to acknowledge that the utilization of high microwave power and extended irradiation time can potentially cause the degradation of specific molecules and subsequently decrease the yield of EO due to excessive heating.

Fig 3B shows the effects between microwave power and SSR on the yields of key lime peel EO at fixed irradiation time (3.5min). As shown in **Fig. 3B**, indicated that there is no perceivable interaction between microwave power and SSR that could affect the EO yield. The observed increase in EO yield was noted when the microwave power was increased from 600 to 1000W. In contrast, the yield of EO exhibited a decrease as the SSR increased from 1:0 to 1:2 w/v. In general, there is a notable interaction between the quantity of water and microwave power, as sample content small amount of water, microwaves are absorbed rapidly resulting in a swift increase in temperature for the sample. Alternatively, sample contains a substantial quantity of water, the absorption of microwaves will occur at a slower rate, potentially resulting in a slower and less uniform heating of the sample [22]. Obviously, the insignificant interaction of microwave power and SSR in this study, could be attributed to the high moisture content in the sample (77.27% ± 0.05). Consequently, the addition of water may result in a slow or uneven heating process in the sample.

Fig 3C demonstrates the mutual effects between SSR and irradiation time on the EO yields extracted at given microwave power (800W). The interaction between pretreatment time and SSR had significantly changed in yield of EO. It was observed that the yield of EO dramatically increased as the pretreatment time increased and slowly decline further increased time. Meanwhile, the yield of EO shown the declined as the pretreatment time increased. The interaction between pretreatment time and the amount of water used shared a similar approach on EO yield. Elongating the pretreatment duration has the potential to enhance the EO yield, but with a threshold limit. Beyond that, additional increments in the duration of pretreatment could potentially result in a reduction in the yield of EOs as a consequence of degradation of the EOs. Similar to a study of Optimization of EO yield from Vietnamese green pepper (Piper nigrum) using hydro-distillation method [23], increasing the ratio of solid material to water from 1:2 to 1:5 (g/mL) has been found to result in a notable enhancement in the extraction of oil yield, with an approximate increase to 0.75%. The elevated concentration of the solution within the range of 1:10 (g/mL) results in a significant reduction in the yield of EO.

According to the analysis of ANOVA and three-dimensional response surface among the three independent variables, the primary factor of microwave pretreatment conditions that affects the yield of EO was the SSR, follow up irradiation time and microwave power.

3.5 Volatile compounds of essential oil

Table 4. presents a comparison of volatile compounds in essential oil (EO) obtained through two methods: without microwave pretreatment and with microwave pretreatment at varying levels of EO yield, namely low, medium, and high. The microwave pre-treatment that resulted in the minimum EO yield was achieved under the following conditions: microwave power of 600 W, irradiation time of 5 minutes, and SSR of 1:0. The optimal conditions for obtaining a medium yield of EO were achieved through the application of microwave pretreatment at a power level of 800 w, a

pretreatment time of 3.5 minutes, and the SSR level of 1:1, and the highest yield of EO were observed under microwave pretreatment at 1000 w of power, duration of 2 minutes, and in the SSR of 1:0. GC-MS was used to identify the volatile components in the extracted oils. Overall, through the application of microwave pretreatment resulted in alterations to certain compounds, including increases, reductions, and the emergence of new compounds.

The study identified a total of 36 volatile compounds in the EO without pretreatment and the lowest yield of EO obtained by microwave pretreatment, and 37 compounds obtained from the medium and highest yield of EO obtained by microwave pretreatment. There were 7 main volatile compounds in the EO of key lime peel, namely D-Limonene (34.595 - 37.13%), β-Pinene (17.495 - 17.772%), γ-Terpinene (8.758 - 10.052%), β-Phellandrene (4.986 - 5.667%), Citral (3.985 - 4.986%), Neral (3.163 - 3.927%), and α-Pinene (3.388 - 3.693%). According to research study of key lime EO also share similar of main compound such as limonene was the highest (58.4%), followed by β-pinene (15.4%), γ-terpinene (8.5%), and citral (4.4%) [24]. The results demonstrate that there are numerous similarities in volatile compounds of EOs under the influence of microwave pretreatment with lowest yield to highest yield and with non-pretreatment. The results obtained from GCMS analysis demonstrate that there were no statistically significant variations in the amount of constituents exhibiting relative peak areas greater than 1.158% with the total percentage of these constituents was 99.99 - 100% in the EO yield obtain microwave pretreatment and with non-pretreatment methods. Under the influence of microwave pretreatment, there was slightly increased of main volatile compounds, γ-Terpinene (1.122%), β-Phellandrene (0.426%), D-Limonene (0.085%) and α-Pinene (0.182%). Whereas, Citral (-0.686%) and Neral (-0.54%) were observed to have slightly decreased. According to the study of comparison of microwave-assisted hydrodistillation and solvent-less microwave extraction of EO from dry and fresh Citruslimon (Eureka variety) peel, shown that there only 2% of the constituents with relative peak areas that show the differences [25].

Table 4. The chemical compositions of key lime peel essential oils were analyzed using GC-MS methodology.

Compound names:	Peak area (%)			
	Fresh peel	Lowest yield	Medium yield	Highest yield
α-Thujene	0.635	0.669	0.69	0.731
α-Pinene	**3.388**	**3.435**	**3.581**	**3.693**
Camphene	0.192	0.204	0.205	0.219
β-Phellandrene	**4.986**	**5.237**	**5.334**	**5.667**
β-Pinene	**17.729**	**17.772**	**17.549**	**17.495**
Myrcene	1.944	2.043	2.033	2.102
4-Carene	0.346	0.371	0.448	0.366
o-Cymene	1.925	1.671	0.69	
D-Limonene	**35.881**	**36.175**	**34.595**	**37.13**
β-Ocimene	1.136	1.281	1.268	1.298
γ-Terpinene	**8.758**	**9.916**	**10.052**	**9.671**
Terpinolene	0.654	0.743	0.746	0.731
Linalool	0.654	0.52	0.559	0.512
(-)-Terpinen-4-ol	0.885	0.613	0.653	0.585
5,8,8-Trimethyl-3-oxatricyclo(5.1.0.02,4)octane	0.173	-	0.205	0.128
L-α-Terpineol	1.193	0.724	0.914	0.823
Decanal	0.558	0.613	0.634	0.585
Nerol	0.346	0.149	0.242	0.183
Neral	**3.927**	**3.38**	**3.618**	**3.163**
Geraniol	0.423	0.204	0.261	0.183
Citral	**4.986**	**4.438**	**4.476**	**3.985**
Neryl acetate	0.346	0.371	0.317	0.384
Geranyl acetate	0.751	0.91	0.746	0.878
(-)-cis-β-Elemene	0.481	0.557	0.615	0.53
Dodecanal	0.231	0.26	0.261	0.256
Caryophyllene	1.367	1.448	1.641	1.499
α-Bergamotene	1.405	1.448	1.772	1.59
Humulene	0.173	0.186	0.224	0.201
Germacrene D	0.385	0.409	0.522	0.439
α-Bisabolene	0.173	0.186	0.224	0.201
α-Farnesene	1.578	1.597	2.051	1.828
β-Bisabolene	1.752	1.783	2.126	1.938
Spathulenol	0.173	0.149		0.183
Ledol	0.154	0.186	0.149	0.165
4-(2,2-Dimethyl-6-methylenecyclohexyl) butanal	0.154	0.167	-	0.146
Levomenol	0.154	0.186	0.149	0.165
Octanal	-	-	0.149	-
cis-β-Farnesene	-	-	0.149	0.146
Verbenol	-	-	0.149	0.201

4. CONCLUSION

The application of microwaves as a pretreatment modality is intended to improve the yield of essential oil (EOs). As the result, the yield of EO obtained by using microwave pretreatment had increased about 19.32 ± 0.01% compare to the yield of EO of non-pretreatment key lime peel. A multiple variables optimization approach applying

Response Surface Methodology (RSM) based on Face-Centered CCD (FCCD) was implemented to optimize the influential parameters affecting the efficacy of microwave pretreatment. The optimum of microwave pretreatment conditions for key lime peel EO were achieved in microwave power of 992.99 W, irradiation duration of 2 min and SSR of 1:0. Irradiation time had the greatest impact on the EO yield of key lime peel followed by solid-to-solvent ratio and microwave power. While, the interaction regression effect showed a noticeable effect between microwave power with irradiation time, and irradiation time with SSR. The GC-MS analysis revealed that there were no statistically significant variations in the constituents of EO extracted from key lime peel, regardless of the yield level microwave pretreatment obtained in the lowest to highest yield compared to the non-pretreated sample. The application of microwave pretreatment can be regarded as an environmentally sustainable technology that presents noteworthy benefits to the traditional hydrodistillation extraction method. These benefits include increased yields, reduced energy consumption, an eco-friendlier approach, and decreased expenses.

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