Comparing Hydrodistillation with Steam Explosion Pretreatment and Conventional Hydrodistillation in Kaffir Lime Oil Extraction

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Abstract

Steam explosion was proposed as a pretreatment method to accelerate the hydrodistillation (SE-HD) of kaffir lime leaves. This research aimed to compare SE-HD and conventional hydrodistillation (HD) in terms of extraction yield, extraction time, chemical composition, and essential oil quality. The extraction of kaffir lime oil with SE-HD was superior with regard to extraction time (35 min. vs 120 min.) and extraction yield (1.356% vs 1.182%). The chemical composition and the quality of the extracted essential oil from both methods were quite similar, indicating the absence of adverse influence from the application of steam explosion as pretreatment. Therefore, SE-HD was a fast and an energy-saving method for extracting essential oil from kaffir lime leaves.

Keywords: essential oil; hydrodistillation; kaffir lime leaves; steam explosion

INTRODUCTION

Kaffir lime (*Citrus hystrix.* DC), from the Citrus family, is a type of lime fruit with a very strong fragrance native to Asia, especially the tropical regions (Butryee *et al.*, 2009). The leaves can be used fresh or dried and stored at freezing temperature. The leaves impart an aromatic, strong, unique, spicy flavor in many dishes. Citronellal is dominant compound in kaffir lime leaf oil (Kawiji *et al.*, 2016) . They are widely used as a condiment in various Thai cuisine like *Tom yum* (Siripongvutikorn *et al.*, 2005). In Javanese, they are used to prepare different spicy curry dishes of fish and chicken, as well as barbeques. Kaffir lime leaf oil possesses some important bioactive compounds, such as antioxidant, antileukemic

(Chueahongthong *et al.,* 2011), antitussive, antihemorrhagic, and antibacterial properties, skin-conditioning agents, and antioxidative stress properties (Waikedre *et al.,* 2010). Therefore, kaffir lime essential oils are a potential flavoring ingredient in food, perfumery, and cosmetic industries.

Thirty-eight constituents are identified in the essential oils of kaffir lime leaf, representing 89% of the 89% essential oil (Loh *et al.,* 2011). The oil is rich in monoterpenes (87%) and other minor components like ß-pinene (10%) and limonene (4.7%). It is characterized by high content of terpinen-4-ol (13.0%), α-terpineol (7.6%), 1.8-cineole (6.4%), and citronellol (6.0%). Another research finds twenty-nine compounds in the essential oils of kaffir lime leaves, and ß-citronellal is the major compound amounting to 66.85% of the total oils (Gök *et al.,* 2015).

Hydrodistillation process has been used in the isolation of essential oils (Gök *et al.,* 2015; Kusuma *et al.,* 2017). The conventional method used for extracting essential oils from plant materials is known to have some advantages, i.e., technically simple and cheap, which warrant its feasibility to be commonly used in industrial essential oil extraction.

Steam explosion is a hydrolytic pretreatment that may release the constitutive components of biomass―increasing the enzyme and solvent accessibility of cellulose (Glasser and Wright, 1998), induce the breakdown of lignocellulosic structural elements by the synergism process of heating and shearing forces resulting in moisture expansion, and open the structure and increase the pore size of the cell wall. Since its early development by Mason (1928), steam explosion has been applied extensively in the conversion of biomass, such as eel grass (Viola *et al.,* 2008), hemp (Sipos *at al.,* 2010), *Salix* (Horn *et al.,* 2011), corn stalk (Guo *et al.,* 2010), sumac fruit (Chen and Chen, 2010), and olive leaf (Juan *et al.,* 2016). During steaming, hemicelluloses are subjected to degradation by autohydrolysis. Pretreatment process like ultrasonic, pulsed electric field, and microwave has been extensively studied for its role in enhancing the oil yield in oil extraction from plants. Pretreatments aim to disrupt the cell wall's materials and enlarge its pore size so that the oil is discharged easily during the extraction process. However, the application of steam explosion as a pretreatment in essential oil extraction by hydrodistillation has not been reported yet. Therefore, this research aimed to compare SE-HD and Hydrodistillation (HD) processes in terms of essential oil yield, physical character, chemical composition, and quality.

Materials and Methods

Plant materials

The samples of kaffir lime (*Citrus hystrix* DC) leaves were collected from a local orchard in Klaten, Central Java Province. After these samples arrived at the laboratory, the mature leaves were sorted out manually based on size and color (i.e., glossy dark green and bright green). The leaves were washed under running tap water and used in the experiments without undergoing a crushing process. The species was collected in March-April 2017.

Steam explosion pretreatment

The experimental unit was set up as shown in Figure 1. Three-hundred gram of fresh kaffir leaves and 33% of water in a 3L reactor were heated at a temperature of 120-130^{o}C and saturated pressure of 1 bar. The explosion was achieved by the sudden release of pressure at the studied temperature. The steam explosion was carried out at a temperature of 120^{o}C-130^{o}C and saturated pressure of 1 bar, and the mass fraction of water was 33%. The steam explosion process in this research was a modification technology from the steam explosion designed by Saeki *et al.* (2017).

Hydrodistillation

The hydrodistillation used a modified Clevenger apparatus. The leaf-water ratio in this research was 1:5, and the distillation was conducted for 2 hours. The collected essential oil was dehydrated with anhydrous sodium sulfate, flushed with nitrogen gas, kept in a colored vial, and stored at 4ºC for further analysis. The extraction process that used steam explosion pretreatment was referred to as SE-HD, while the one using untreated kaffir lime leaves was labeled with HD. The oil was analyzed for their specific gravity, refractive index, optical rotation, and appearance. The water content of fresh kaffir leaves was 72.612±0.021%. The essential oil yield was calculated using the following formula (Kasuan *et al.*, 2013; Kusuma *et al.*, 2017).

$$Yield\ \left(\%,\frac{w}{w}\right) = \frac{mass\ of\ essential\ oil}{mass\ of\ fresh\ leaves \times (1 - water\ content)} \times 100\% \quad (1)$$

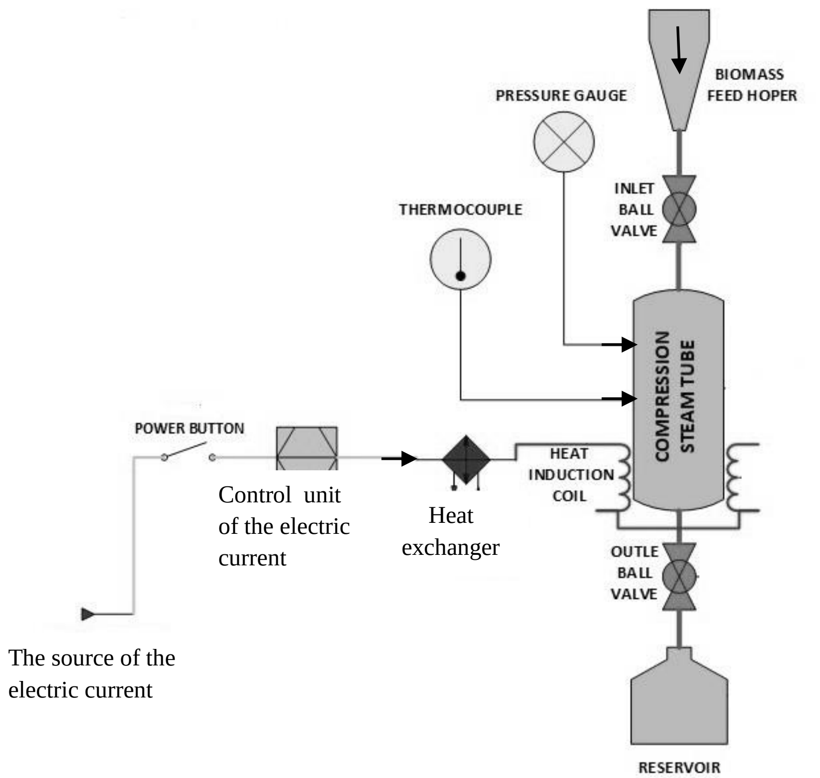

Figure 1. The experimental setup of steam explosion

Chromatographic analysis

The chromatography analysis was performed using a gas chromatograph-mass spectrometer (SHIMADZU-QP2010S) that was equipped with a fused-silica capillary column

SH-Rxi-5Sil MS 30 m x 0.25 mm (ID) x 0.25 µm (film thickness). The injection temperature was 260^{o}C with an injection volume of 0.3 µL in the split mode (a split ratio=10:1). The oven temperature was programmed at 40^{o}C for 0.5 min, followed by an increase of 5^{o}C per minute up to 250^{o}C. The carrier gas was Helium (3.0 mL/min). These analyses were carried out using the Hewlett-Packard 5890A GC/MS. The mass spectra were obtained by electron ionization (EI). The temperature of the ion source was 280^{o}C and the electron energy was 70eV. The volatile compounds were identified by comparing their mass spectra with the mass spectral database.

Leaf structure observation

The scanning electron micrographs of *Citrus hystrix* leaves were captured using FEI Quanta 200. Before SEM evaluation, the samples were coated with gold using the plasma sputtering technique. The micrographs were carried out using a field-emission SEM (JOEL JSM-6510 LA, Japan). The structures of the fracture surface were observed under the same magnification.

Statistical analysis

Analysis of variance (ANOVA) in a completely randomized design, Duncan's multiple range test was performed to compare the data. All determinations were done at least in twice and all were averaged. The confidence limits used in this study were based on 95% ($p < 0.05$).

RESULTS AND DISCUSSION

Essential Oil Extraction

The yields of SE-HD and HD essential oil extraction from fresh kaffir lime leaves were 1.386 ± 0.09% and 1.256 ± 0.08%, respectively, after 2-hour extraction (Figure 2). The extraction time for the SE-HD method was shorter than the conventional HD. The time required to achieve maximum yield was 35 min for the SE-HD method and 120 min for the HD method. The results revealed a substantial saving of time and energy.

Figure 2a and 2b show that the extraction yield varies according to extraction time. There were four phases observed in this experiment. The first was the heating phase from room temperature to boiling temperature in which the first droplet of essential oil was seen. Compared to the HD method, the heating process in SE-HD required only 5 min instead of 15 min. With this extraction time, SE-HD produced a higher oil yield (10.46 %) than HD. The steam explosion, as seen in Figure 5, could induce the breakdown of the structure of the lignocellulosic matrix in leaves (Viola *et al.*, 2008; Juan *et al.*, 2016). Therefore, the trapped essential oils might be removed easily from the matrices. The second phase was represented by the first exponential quantities of extracted essential oil, which accounted for approximately 80% of the total yield obtained after 25 min in the HD method and 10 min in the SE-HD method. In this step, the oil came out of the epidermis on the leaves and, transported by the steam. The third phase was indicated by the second exponential quantities of extracted essential oil, which represented the internal diffusion of the essential oil from the middle of the leaf matrices to the external medium. This stage was achieved after 35 min and 120 min by SE-HD and HD, respectively. The extracted oil constituted nearly 20% of the total yield. The fourth step (step 4) was the end of the extraction processes. Since almost all of the essential oil was extracted in the previous stages, there was no additional yield observed at the final stage

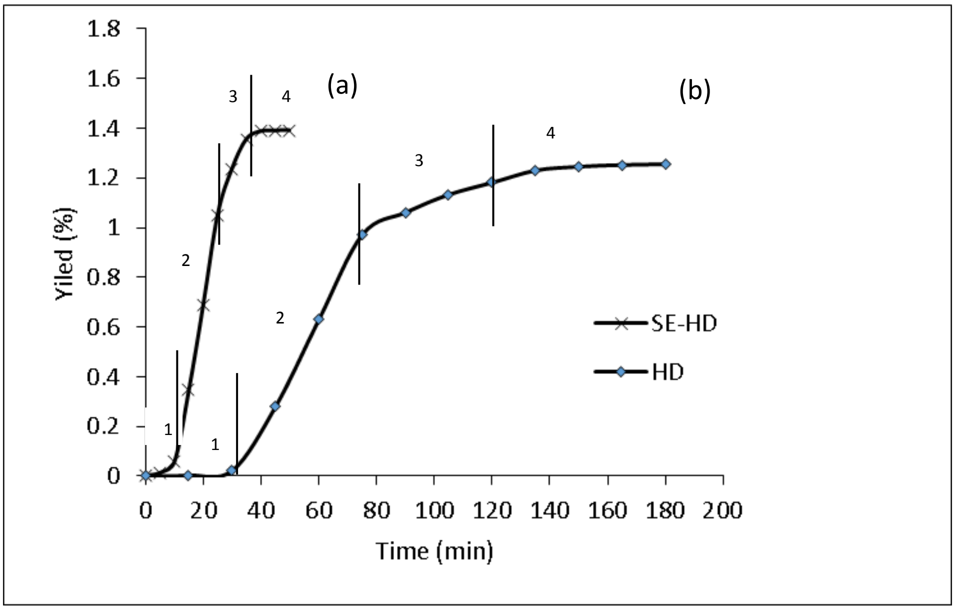

Figure 2. The yield profiles as a function of time for the essential oil extraction from fresh kaffir lime leaves using SE-HD (a) and HD (b).

The variation of yield by extraction time was determined using kinetic modeling (Kusuma *et al.*, 2017). According to Figure 2, the kinetic models for the extraction of kaffir lime leaf oil by SE-HD and HD methods were developed using the first- and second-order models. The linearization of each plot of the experimental data was based on slope and intercept. From this process, the rate constant (k_{1}), extraction capacities (C_s), and coefficient of determination (R^2) for the first-order kinetic model were determined. The same data and procedure were used to determine the values of k_2, C_s, and R^2 for the second-order kinetic model. The linearization of the first- and second-order kinetic models for the extraction of kaffir lime leaf oil using HD and SE-HD methods is shown each in Figure 3 and Figure 4.

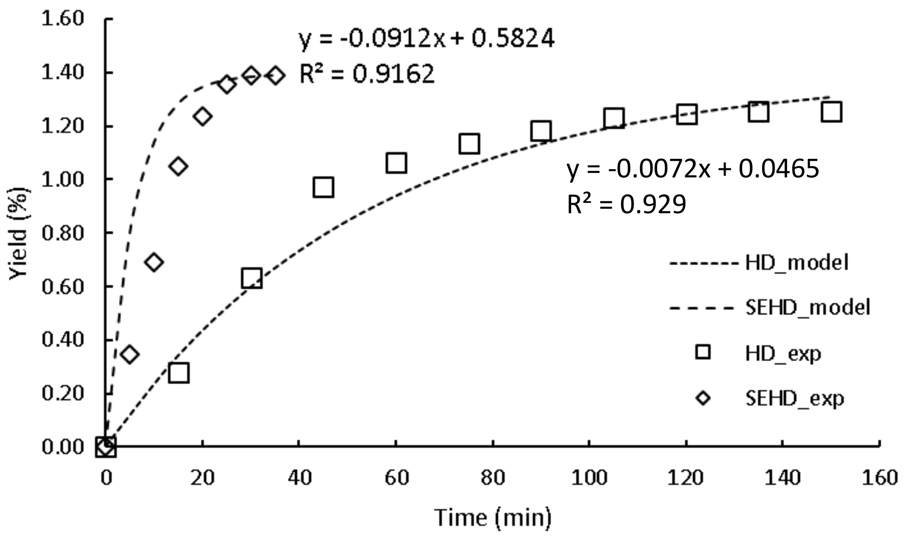

Figure 3. The linearization of the first-order kinetics of kaffir lime leaf oil.

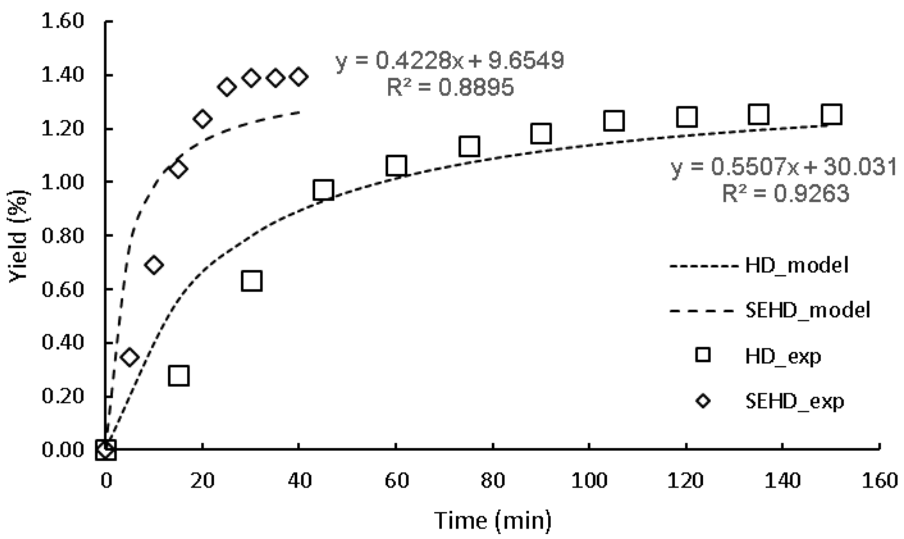

Figure 4. The linearization of the second-order kinetics of kaffir lime leaf oil.

Table 1 shows that the first-order rate constant (k_1) for the SE-HD-assisted extraction is higher (i.e., by 9.0695 times) than the HD-assisted one. This condition indicates that SE-HD facilitates a faster oil removal than HD. This research also evaluated the experimental results from these extraction processes in the second-order kinetic model. The k_2 values of the extraction processes have the same trend as the first-order kinetic model. When compared with the HD-assisted extraction, SE-HD results in a faster rate constant of oil extraction by 5.4025 times. Therefore, the extraction of kaffir lime leaf oil using the classic HD requires longer extraction time when compared with the SE-HD method. The study results indicated SE-HD as a more favorable method for extracting the essential oil of kaffir lime leaves than HD. Since SE-HD does not use any chemicals, this method can be considered as an environmentally friendly extraction process.

Table 1. The linearization of the first- and second-order kinetic models for the extraction of kaffir lime leaf oil using HD and SE-HD method.

Extraction methods	Slope[a]	Cs, g L^{-1}	Intercept[a]	k_1, min^{-1}	k_2, L $g^{-1}min^{-1}$	R^2
HD	-0.0072	1.256	0.0465	0.0187	-	0.9290
SE-HD	-0.0912	1.392	0.5824	0.1696	-	0.9162
HD	0.5507	1.256	30.0310	-	0.0318	0.9263
SE-HD	0.4228	1.392	9.6549	-	0.1718	0.8895

[a]Values generated from the feature Trendlines in Microsoft Excel (see Figure 3 and 4)

The Physical Properties of Kaffir Lime Leaf Oil

The physical properties of the essential oil extracted from kaffir lime leaves using SE-HD and HD are shown in Table 1. The results seemed to indicate that there was no significant difference among the physical properties of SE-HD and HD essential oil, except for color. The SE-HD essential oil has a lighter color than that of HD essential oil. The characteristics of these oils are quite similar to the kaffir lime leaf oils treated with automated steam distillation process before hydrodistillation (Kasuan *et al.,* 2013). Therefore, based on the physical properties, yield, and extraction time, the steam explosion was the appropriate pretreatment method before the hydrodistillation process for kaffir leaf oil extraction. The SE-HD-assisted extraction produces oil with favorable appearance (i.e., colorless) and high yield, and it may save the distillation time.

The quality standards for the physical properties of essential oil from kaffir lime leaves are not available yet. However, the quality standards for kaffir lime peel oil have been issued, namely, TCFF Code: 2003-40020. Based on the previous research (Khasanah *et al.,* 2015), hydrodistillation with aging pretreatment for the extraction of kaffir lime leaf oil produces 0.867±0.029% of essential oil (i.e., oil yield) with the following properties: specific gravity of l 0.843±0.0005, a refractive index of 1.451±0.0001, and an optical rotation -13.5^{0}. The physical properties of the kaffir lime leaf oil in this research are nearly similar to the results of the other studies, except for the oil yield. Using SE-HD as a pretreatment has increased the oil yield by approximately 1.606 times. As a conclusion, this procedure proposed in this research yields essential oil with similar quantity but better quality than the previous studies.

The Characteristics of the Volatile Compounds of Essential Oils

The chromatograms of SE-HD and HD essential oils are similar (Figure 5 and Figure 6) with citronellal (peak number 9) as the most prominent compound (i.e., over 70% of total chromatogram), followed by citronellyl acetate, citronellol, linalool, geranyl acetate, nerolidol, and other compounds with small concentration. In this study, the application of steam explosion as a treatment before the hydrodistillation process, which may profoundly accelerate the extraction process, did not cause any considerable changes in the volatile oil composition (Table 2). The presence of citronellal as the main component is also reported in many previous studies (Loh *et al.*, 2011; Khasanah *et al.*, 2015; Li *et al.*, 2011). There were five groups observed in both SE-HD and HD essential oil. Monoterpenes were the most abundant volatile compounds in kaffir lime leaf oil from the HD and SE-HD extraction-contributing to 70% of the total volatile content, followed by esters (15%), acid (5%), phenol (5%), and alkaloid (5%). The terpenoids contained in the kaffir lime leaf oil were monoterpene hydrocarbons (ß-phellandrene, ß-pinene, trans-ß-ocimene), monoterpene alcohol (cis linalool, trans-linalool oxide, Linalool, Citronellol), oxygenated monoterpenes (Citronellal, Isopulegol), cyclic monoterpenes (3-cyclohexane-1-ol), sesquiterpenes (Cadinene, Nerolidol), and sesquiterpene hydrocarbons (γ-terpinene, ß-caryophyllene). Furthermore, this research found three esters in the volatile constituents (citronellyl acetate, neryl acetate, geranyl acetate), one acid (octadecanoic acid), one phenol (eugenol), and one alkaloid (pyridine) were considered.

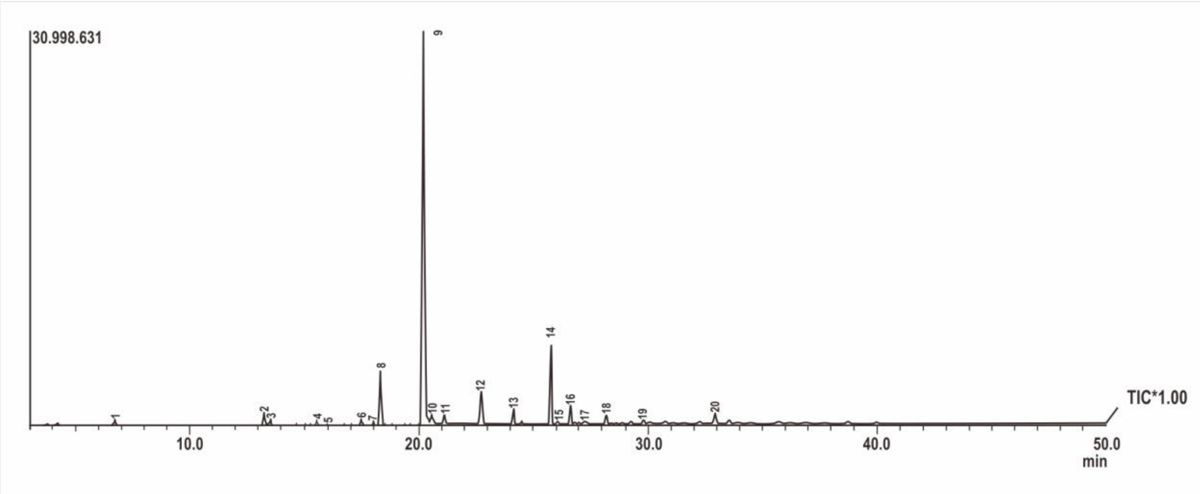

Figure 5. The GC-MS Chromatogram of kaffir lime leaf oil obtained by SE-HD

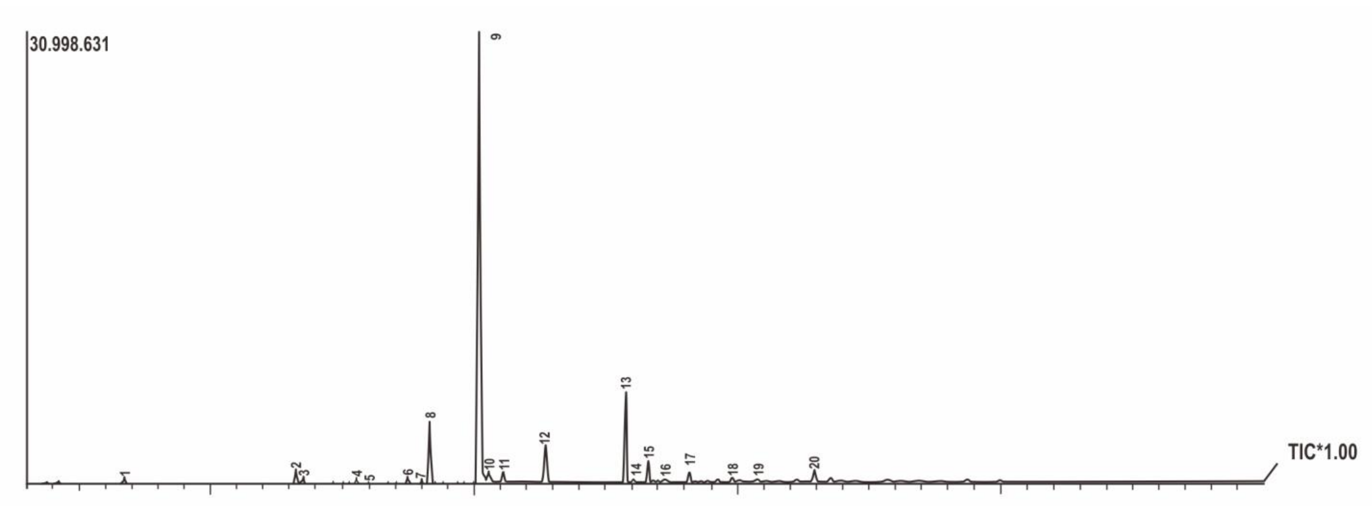

Figure 6. The GC-MS Chromatogram of kaffir lime leaf oil obtained by HD

Table 2. The physical properties of the essential kaffir lime leaf oil extracted with SE-HD and HD

Physical properties	Essential oils	
	SE-HD	HD
Specific gravity (22^{o}C)	0.855±0.12^{ns}	0.848±0.23^{ns}
Refractive index (25^{o}C)	1.447±0.13^{ns}	1.445±0.14^{ns}
Optical rotation in degree	-12.5±0.00	-12.5±0.00
Appearance	Colorless	Pale

Column with different letters represents a significant difference ($p < 0.05$, ns: not significant)

The major constituents of the volatile compounds of kaffir lime leaf oils, which are presented in Table 3, are also found in other essential oils. Monoterpene hydrocarbon like ß-phellandrene is a major compound found in oils extracted from *Curcuma longa* roots and rhizomes (Li *et al.*, 2011). This compound is also found in coriander and lemon essential oils with potent bioactivity as insecticide and antimicrobial (Teixeira *et al.*, 2013). Trans-ß-ocimene is also found in the flower, peel, and leaf of some citrus species (e.g., mandarin, orange, and pummelo). Ocimenes can change into 2,6-dimethyl-3,5,7-octatriene-2-ol, Z,Z-because of oxidation process (Sonwa *et al.*, 2007). Ocimenes are also found in the spice ajwain (*Trachyspermum ammi*) with some potential bioactivities, such as anti-inflammatory, anticancer, antibacterial, antitumor, insecticide, sedative, pesticide, herbicide, immunomodulator, fungistat, antiobesity, and seasoning 3-cyclohexane-1-ol is a cyclic monoterpene compound found in *Citrus limetta* (Juáréz *et al.*, 2012). ß-caryophyllene and nerolidol are also found in the essential oil of cinnamon (*Cinnamomum osmophloeum*) twigs, and they exhibit excellent anti-inflammatory activity (Tung *et al.*, 2008). Isopulegol is a monoterpene compound with a fresh green odor (Paramasari, 2017). Esters are considered as a key aroma constituent because they impart a fruity aroma. Esters like citronellyl acetate create many types of fruity and floral scents and, therefore, they are suitable as flavor ingredients.

The Leaf Structure of Kaffir Lime Leaves

Kaffir lime leaves constitute biomass of lignocellulosic composition, a complex mixture of cellulose, hemicelluloses, and lignin. Steam explosion pretreatment allows the breakdown of biomass components by steam heating and shearing forces due to the concomitant expansion. The sudden decompression, which terminates the reaction, decreases the temperature rapidly and, therefore, damages the particulate structure of kaffir lime leaves. The distinct physical change of SE-HD and HD kaffir lime leaves is shown in Figure 5a-c.

Table. 3. The characteristics of the volatile compounds of kaffir lime essential oil extracted with SE-HD and HD

Peak no.	Compounds	T_r (min)	% Relative Peak Area		*ref	Odor characteristics
			SE-HD	HD		
1	Pyridine	6.738	0.40	0.35	-	
2	ß-phellandrene	13.231	1.02	1.77	[a]), [b])	Terpeny, fruity, minty, herbaceous
3	ß-pinene	13.531	0.50	0.75	[a]), [b]), [c])	Musty, green, sweet, pine-resin like, pungent (turpentine), woody
4	Trans-ß-ocimene	15.537	0.47	0.56	[a]), [b])	Herbaceous, mild,typical odor of citrus, orange, or lemon
5	Cadinene	29.809	0.25	0.18	[b])	Herbaceous, woody
6	Nerolidol	32.907	1.30	1.43	[c])	Waxy
7	γ-terpinene	16.003	0.12	0.13	[a]), [b]), [c])	Citrus-like, herbaceous, fruity, sweet
8	ß-caryophyllene	28.175	0.81	0.31	[e]), [c]), [d])	Oily, fruity, woody
9	Cis linalool	17.456	0.54	0.60	-	
10	Trans-linalool oxide	18.015	0.26	0.28	[b])	Fresh, green, fruity
11	Linalool	18.317	5.07	4.94	[b]), [c]), [d])	Green, floral, sweet, the typical odor of lemon or lavender
12	Citronellol	22.704	4.93	5.79	[a]), [f]), [g])	Sweet, citrus-like
13	Citronellal	20.223	72.26	70.99	[a]), [f]), [g])	Citrus-like
14	Isopulegol	20.555	1.31	0.88	[i])	Fresh green
15	Citronellyl acetate	25.769	6.87	0.20	[a]), [f]), [g])	Citrus-like, oily
16	Neryl acetate	26.096	0.18	1.55	-	
17	Geranyl acetate	26.630	1.51	0.18	-	
18	3-cyclohexen-1-ol	21.107	0.87	0.73	-	
19	Octadecanoic acid	24.128	1.23	7.33	-	
20	Eugenol	27.230	0.11	1.06	[g])	Herbaceous

[*]The reference are obtained from previous studies. [a]) Gonçalves *et al.*, (2014), [b]) Choi (2003), [c]) Njoroge *et al.*, (1994), [d]) Choi and Sawamura, (2000), [e]) Sato *et al.,* (1990), [f]) Tung *et al.*, (2008), [g]) Paramasari (2017), [i]) Juáréz *et al.*, (2012)

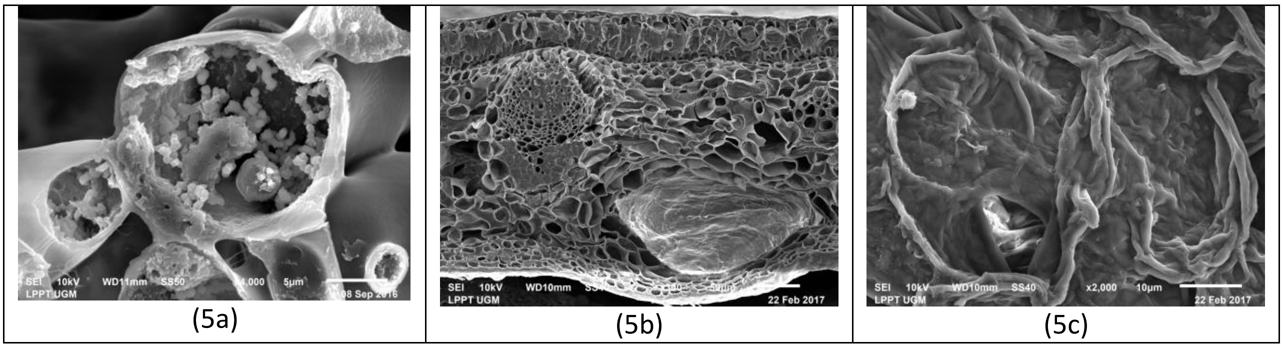

Figure 5. The scanning electron micrograph of kaffir lime leaves: (a) untreated, (b) after hydrodistillation and (c) after steam explosion-hydrodistillation

The micrograph of the untreated leaves (Figure 5a) shows that the cell structure~~s~~ differ somewhat significantly from the treated leaves, as presented in Figure 5b (HD) and 5c SE-HD). The structure of the untreated kaffir lime leaves (Figure 5a.) clearly show that the shape of the glandular cells is rigid and that their volatile oils are still intact. Figure 5b shows that after HD extraction, the glandular cell is empty and collapsed, the essential oil is completely discharged, but the cell is still intact. Figure 5c shows that the SE-HD kaffir lime leaves processed by a combination of steam explosion and hydrodistillation suffer massive damage not only on their external surface but also on their internal surface (e.g., the cellular materials). These findings indicate that steam explosion makes the glandular cell disintegrate or rupture more rapidly and efficiently, which is similar to the previous research that employs steam explosion for the extraction of sugars and natural antioxidants from olive leaves (Juan *et al.*, 2016). The cellular damage in kaffir lime leaves makes the oil flow out quickly from the cell and, thereby, shortens the distillation time, as shown in Figure 2. As a result, the essential oil in this study was removed at a fast rate from the glandular cells.

CONCLUSIONS

Steam explosion pretreatment causes the kaffir lime leaf cell to collapse and rupture. It reduces the extraction time, resulting in the same quality as the untreated kaffir lime leaves but with a higher yield. Therefore, SE-HD is recommended as an excellent alternative method for the extraction of essential oils. Furthermore, as an extraction method, it has many favorable characters, namely fast, modern, and environmentally friendly.

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