Effects of drying time on essential oil production of kaffir lime (Citrus hystrix DC) leaves at ambient temperature

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Abstract. Drying was one of the primary processes in essential oil production. Time factor in drying affects both the content and chemical compositions of essential oils. This study aims to evaluate the influence of the drying time of kaffir lime leaves on the yield at room temperature and the major volatile compounds in the essential oils by observing day-to-day treatments. As a key odorants in essential oils, the quantity of citronellal was determined by Gas Chromatography (GC) using the standard compound. Based on the daily observation results, a long-time drying gave a significant effect on the yield and the major volatile compounds. The highest yield 1.26±0.07 % (w/w) was obtained on the fourth day, while the highest content of citronellal compounds, 87.58±0.33% was achieved on the fifth day of drying. A prolonged drying resulted in a negative effect on not only the quantity, but also the quality of essential oils. Accordingly, this study suggests that the drying technique at room temperature best lasts for four until five days to obtain the most favorable quality and quantity of essential oils.

1. Introduction

Essential oils from kaffir lime (Citrus hystrix. DC) leaves are natural flavoring materials of commercial importance. Essential oil is the secondary metabolite from this plant. Kaffir lime leaves oil possesses some important bioactivities; such as antioxidant [1], antileukemic [2], antitussive, antihemorrhagic, antioxidative stress properties [3], and antibacterial properties [4]. The enantiomer of citronellal in kaffir lime leaves oil is responsible for odor characteristic of these leaves. Twenty-nine compounds are found in the essential oils of kaffir lime leaves [5]. Furthermore, β-citronellal is the major compound, amounting to 66.85% of the total yield.

Essential oils are natural products obtained from different parts of plants by extraction and distillation process. Many studies had examined the effects of different drying methods on the content and chemical compositions of essential oils. For instance, extraction on dried leaves both reduced the moisture content and affected the volatile compounds of the produced essential oils. Although the concentration of the
volatile compounds tends to change during the process, its magnitude depends on the type of the product, drying methods and conditions [6]. Meanwhile, drying methods also can affect both oil content and the composition of aromatic plants [7],[8],[9]. [10] investigated the effect of different temperature on the aromatic profile of fresh ginger in microwave-drying process. [11] studied the effect of freeze-drying time on the levels of the two main components of fennel essential oil. Higher drying temperature decreased the content of Artemisia annua essential oil [12]. Meanwhile, air drying at ambient temperature and infrared-drying at 45°C significantly increase the content of the essential oil of Laurus nobilis L. leaves [13]. Interaction between different temperatures and various air flow rates in drying condition considerably affected both content and chemical composition of the essential oil of lemon verbena (Lippia citriodora) [14].

Citronellal is the major aromatic profile in kaffir lime leaves oils, and the highest value of essential oil depends on it. The drying method is proven to significantly influence the quality of the content and composition of aromatic plants. Therefore, this study aims to investigate the major volatile compounds of kaffir lime leaves that had been subjected to different drying processes.

2. Experimental

2.1. Materials
Samples of kaffir lime leaves were collected from a local orchard in Klaten, Central Java. After the samples arrived in the laboratory, the mature leaves were sorted out and divided into nine groups, each of that received different treatments during the drying process. All chemicals and solvents used in this research were of analytical grade and procured from Merck. The citronellal standards (C_{10}H_{18}O) were procured from Santa Cruz Biotechnology.

2.2. Drying treatment
Kaffir lime leaves were dried at a room or ambient temperature (i.e., averagely 25±1°C). About 300g of the sample were placed on a stainless steel mesh with known weight and volume. In all experiments, the samples had the same bed height and were spread as a thin layer with a bulk density of 300 g/11.25 cm³. A similar drying temperature is also preferred in the drying of lemon Myrtle leaves [7]. During the drying process, the samples were regularly weighed at a fixed time interval for eight days. The weighing process used a digital balance with 0.01 accuracy and 1,500 g capacity.

2.3. Determination of moisture content
Moisture content of the leaves was determined by a gravimetric method using a laboratory-scale drying chamber by specifying the initial wet basis and final dry matter content in a sample that had been dried at a temperature of 105°C for three hours. The results were presented in mean values [9].

2.4. Essential oil isolation
The essential oil was isolated by using hydrodistillation method with the ratio of leaves to water 1:5. The distillation was carried out until the maximum possible quantity of oil was obtained (i.e., two hours). The yield was determined based on dry weight. The essential oil was collected in numbered and colored vials, dehydrated with anhydrous sodium sulphate, capped under nitrogen, and kept at 4°C for preservation until the next analysis.

2.5. Citronellal determination
Gas Chromatography (SHIMADZU-QP2010S) method was used to determine the citronellal content. GC analyses were performed using a Hewlett-Packard 5890A apparatus equipped with a fused silica capillary column (SH-Rxi-5Sil MS 30 m x 0.25 mm ID x 0.25 μm film thickness). These analyses used the following temperature program, i.e., oven isotherm at 40°C for 10 minutes followed by a temperature increase from 40°C to 280 °C at a rate 4°C/min and isotherm at 280 °C for 10 minutes. Injector and detector (FID) temperatures were both set at 290 °C. Helium, with a linear velocity of 32 cm/s was used.
as the carrier gas. Percentages of the compounds were calculated by the area normalization method without considering the response factors. A total of 2.0 µL diluted samples was injected in the split mode (1:60). The volatile compounds were identified by comparing their mass spectra with those of the mass spectral database [14]. Components of essential oil were identified by calculating their retention indices under a temperature-programmed condition for C_{10}H_{18}O and comparing them with the bibliographic data, whereas individual compounds were identified by comparing their mass spectra with those of the authentic compounds in the mass spectral reference library, and the results were confirmed by comparing their retention indices with the authentic compounds.

2.6. Statistical analysis
The data were the average values of the three samples presented in mean ± standard deviation. The statistical analysis was performed in STATISTICA. Differences were tested for their significance by using analysis of variance (ANOVA) at a significance level of P< 0.05.

3. Results and Discussion

3.1. Drying time and moisture content
Fresh kaffir lime leaves had moisture content of 72.61% (wb). Influence on drying process on moisture content in eight days treatment are presented in Table 1. Drying process was simultaneous heat and mass transfer. The rate of moisture loss in drying was assumed to be proportional to the moisture remaining to be lost [15]. Based on this research, after four-day drying dehydration process was slower than that on the days before. The phenomenon was influenced by the movement of moisture occurring by diffusion and was analogous to that of heat conduction in material. The drying constant of the process achieved on equilibrium moisture content, increasing drying time did not decrease the moisture content.

| Time (day) | Moisture content (%) |
|------------|----------------------|
| 0          | 72.61±0.02<sup>c</sup> |
| 1          | 71.14±0.03<sup>cd</sup> |
| 2          | 70.19±0.01<sup>c</sup> |
| 3          | 68.79±0.72<sup>bc</sup> |
| 4          | 67.01±0.01<sup>bc</sup> |
| 5          | 66.80±0.01<sup>b</sup> |
| 6          | 65.99±0.04<sup>a</sup> |
| 7          | 65.55±0.02<sup>a</sup> |
| 8          | 64.83±0.03<sup>a</sup> |

<sup>a</sup>Means±SD; n=3. Values in same row marked by the different letters are significantly different  p<0.05

In general, there were three important stages in a drying process: The first phase or initial period happens when sensible heat was transferred to the product from the inlet to the process condition. The rate of evaporation would increase dramatically during this period with mostly free moisture content being removed. During the second phase or constant period, free moisture persists on the surfaces and the rate of evaporation altered very little as the moisture content reduced. There was a gradual and relatively small increase in the product temperature during this period. Finally, the third phase or falling rate period was the phase when migration of moisture from the inner interstices of each particle to the outer surface become the limiting factor that reduced the drying rate.
3.2. Volatile oil yield

The yield of kaffir lime essential oil produced in this experiment was monitored by taking a daily reading on oil yield for eighth days. It was then calculated on a wet basis and the experiment was repeated three times for every different treatments drying. After accumulated in percentage during the extraction process at different treatments drying, it was tabulated in Table 2. The yield of essential oils of fresh leaves was 0.56±0.06 %. Although the fresh leaves and leaves with the drying treatment on first day was not significantly different, the yield tended to increase until the fourth day of drying time. Furthermore, increasing drying time would reduce the yield value. Hence, the yield of the essential oils of dried leaves were not significantly different on either the second or eight day.

**Table 2. Effect of different drying time on the yield of kaffir lime essential oil**

| Time (day) | Essential oil yield (%) |
|------------|-------------------------|
| 0          | 0.56±0.06^a             |
| 1          | 0.62±0.02^a             |
| 2          | 0.99±0.14^bc            |
| 3          | 1.05±0.07^bcd           |
| 4          | 1.26±0.07^d             |
| 5          | 1.18±0.06^cd            |
| 6          | 1.05±0.04^cd            |
| 7          | 0.97±0.01^bc            |
| 8          | 0.88±0.03^b             |

^aMeans±SD; n=3. Values in same row marked by the different letters are significantly different  \( p<0.05 \)

Table 2 shows that different treatment at long-day drying gave a significant effect on the essential oil content of kaffir lime leaves (\( p < 0.05 \)). These results were in agreement with those numerous studies confirming that the yield essential oil was affected by different drying conditions [7, 8, 12, 16]. Our findings showed that increased drying time (from zero to four days) resulted in a significant increase in the essential oil content. Meanwhile, the decreased amount of yield due to increased long time drying might be caused by oil accumulated in storage cells of essential oil at different developmental stages [17]. Drying was simultaneous process of heat and mass transfer that affected schizogenous oil glands in kaffir lime leaves. Essential oil is a volatile compound, therefore the damage of oil gland cells due to drying time process of kaffir lime leaves could reduce essential oil yield by hydrodistillation methods. Similar observations were made in the previous work with peppermint [18,19] demonstrating that the accumulation of leaf essential oil correlated with the developmental distribution of glandular trichomes.

3.3. Characterization of citronellal compounds in kaffir lime essential oil

Volatile oil obtained from kaffir lime leaves that had been subjected to treatment with different durations of drying was analyzed with GC. This experiment identified that citronellal was the major compounds of essential oil in *Citrus hystrix* leaves. Effects of drying methods on the citronellal content in essential oil are shown in Table 3. Findings clarified that drying the leaves influenced the chemical composition of the essential oil. The level of citronellal increased while the drying time increased. The highest retention level was obtained on the fifth day, i.e., 87.58%, and the level of citronellal decreased afterward.

The essential oil of kaffir lime leaves significantly decreased after five days of drying. It was importantly noted that the effects of drying time on volatile compounds and oil content were similar. In another research, cavity filling in *Citrus* peel appeared to be a relatively slow process when compared with the accumulation of essential oil in the glandular trichomes of peppermint. Genes encoding all previously characterized terpene syntheses of *Citrus* peel are expressed at high levels in the resultant essential oil [17]. Drying process before essential oil extraction aimed to reduce the moisture content.
During this process, the volatile compounds were altered. Previous research had successfully proven that the concentration of volatile compounds were always affected in this process, the magnitude of that depended on the type of the product, drying methods, and conditions [6].

**Table 3.** Effect of different drying time on the citronellal compounds of kaffir lime essential oil

| Time (day) | Citronellal compounds (%) |
|-----------|---------------------------|
| 0         | 44.79±0.02^a              |
| 1         | 47.87±0.08^b              |
| 2         | 57.14±0.02^c              |
| 3         | 63.18±0.54^d              |
| 4         | 72.12±0.19^g              |
| 5         | 87.58±0.33^i              |
| 6         | 85.97±0.21^h              |
| 7         | 69.88±0.70^f              |
| 8         | 67.44±0.39^e              |

^aMeans±SD; n=3. Values in same row marked by the different letters are significantly different  \( p<0.05 \)

**4. Conclusion**

Essential oil yields tended to increase in kaffir lime leaves that have been dried for four-five days at an ambient temperature. Longer drying period had been proven to decrease the yield. The presence of citronellal as the volatile and major compound of essential oils signified the quality of the yield. Citronellal gave a specific flavor to kaffir lime leaves. Finally, this research concluded that the drying period of kaffir lime leaves at an ambient temperature lasted no longer than five days.

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**References**

[1] Hutadilok-Towanata N, Chaiyamutti P, Pathong K, Mahabusarakam W, and Rukachaisirikul V 2006 *Pharm. Biol.* **44** 221-228

[2] Ampasavate C, Okonogi S, and Anuchapreeda S 2010 *Afr. J. Pharm. Pharmacol.* **4** 13-21

[3] Laohavechnich P, Muangnoi C, Butryee C, and Kriensinyos W 2010 *Science Asia* **36** 112-117

[4] Siripongvutikorn S, Thummaratwasik P, and Huang Y W 2005 *Tom-Yum LWT* **38** 347

[5] Loh F S, Awang R M, Omar D, and Rahman M 2011 *J. Med. Plant. Res.* **5** 3739-3744

[6] Barbieri S, Elustondo M, and Urbicain M 2004 Journal of Food Engineering, **65** 109-115

[7] Buchaillot A, Caffin N, and Bhandari B 2009 *Drying Technology* **27** 445-450

[8] Huang B, Wang G, Chu Z, and Qin L 2012 *Drying Technology* **30**(3) 248-255

[9] Antal T, Figiel A, Kerekes B, and Sikolya I 2011 *Drying Technology* **29**(15)1836-1844

[10] Hussain A, Li Z, Ramanah DR, Niamnuy C, and Raghavan G S V 2019 *Drying Technology* **28**(1) 42-48

[11] Gardeli C, Evageliou V, Poulos C, Yanniots S, and Komaitis M 2010 *Drying Technology* **28** 542-549

[12] Khangholi S H and Rezaeinodehi A 2008 *Pakistan Journal of Biological Science* **11**(6) 934-937

[13] Sellami I H, Wannes W A, Bettaieb I, Berrima S, Chahed T, Marzouk B, and Limam F 2011 *Food Chemistry* **126** 691-697

[14] Shahhoseini R, Ghorbani H, Karimi S R, Estaji A, and Moghaddam M 2013 *Drying Technology* **31** 1020-1028

[15] Phuongchandang S, Srinukroh W, and Leenanon B 2008 *Drying Technology* **26** 1602-1609

[16] Asekum O T, Grierson D S, and Afolayan A J 2007 *Journal of Applied Sciences* **7**(7) 1005-1008
[17] Voo S S, Howard D, Grimes, and Lange B M 2012 *Plant Physiol.*, **159** 81-94
[18] Turner G W, Gershenzon J, and Croteau 2000 *Plant Physiol.*, **124** 665-680
[19] Rios-Estepa R, Lange I, Lee J M, and Lange B M 2010 *Plant Physiol.*, **152** 2015-2119